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MEMOIRS
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CONTRIBUTION TO THE STUDY OF
THE HYPOPHYSIS CEREBRI WITH
ESPECIAL REFERENCE TO ITS
COMPARATIVE HISTOLOGY

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THE HYPOPHYSIS CEREBRI

1. HISTORICAL

Since Marie first suggested an etiologic relation between acromegaly and the hypophysis cerebri, the problem of this organ has increasingly engaged the attention of medical investigators. Extracts of the hypophysis have been employed therapeutically, experimental researches have dealt with many phases of the question, clinical observations have been controlled by pathological investigations; but there is as yet no unanimity in the opinions expressed as a result of experimentation or clinical observation, the therapeutic indications are not yet as well defined as might be desired, and as a result this therapy is still open to the charge of empiricism. In consequence, the real limits of our knowledge in this question are still somewhat obscured. It is, perhaps, opportune in this light to review the facts established concerning the hypophysis and at the same time take into consideration the contentions which have arisen about it. For convenience, the work of previous investigators may be grouped under three general headings: (1) Morphological Research; (2) Experimental Research; (3) Clinical and Pathological Observation.

MORPHOLOGICAL RESEARCH

The pituitary body was known to the ancients as a glandular structure which supplied a mucous secretion to the membrane of the nose (pituita—mucus). Vesalius¹ called it the *glans pituitaria excipiens*. The term *hypophysis cerebri* was first applied by Soemmering² in 1778. Chaussier³ referred to it as the *appendice sus-sphenoidal du cerveau*. It was also known as the *colatorium* and *sentina*. The terms *hypophysis* and *pituitary body* are often used synonymously. To avoid any possible confusion, the suggestion of Collina⁴ will be followed here, designating the epithelial lobe the *pituitary gland*, the neural lobe the *processus infundibuli*, and the entire body the *hypophysis*.

Origin of the hypophysis

Investigators have been divided on the question of origin of the hypophysis. Some, particularly among the early workers, held that the organ was a modified portion of the brain, arising from the hind end of the interbrain and infundibulum. Joh. v. Müller⁶ and Stannius⁷ were of this opinion for fish, amphibians and reptiles. Hushke⁸ regarded it as the cephalic extremity of the spinal cord situated in immediate relation to the infundibulum. von Bauer⁹ observed a protuberance in the chick near the infundibulum which subsequently formed the hypophysis. Schmidt¹⁰ described it as arising from the floor of the diencephalon, at first in communication with that vesicle but later cut off from it.

Several investigators have maintained that the hypophysis had its origin entirely in the cephalic end of the chorda dorsalis. Reichert¹⁶ and His¹⁷ were at one time, of this opinion. Reichert¹⁹ in a later work, claimed that the pineal gland and, perhaps, the hypophysis were outgrowths of the pia mater. Subsequently, His also took a different view as to the origin.

A third, and by far the largest, group has held that the origin of the hypophysis was two-fold, one part from the mouth cavity, the other from the infundibular region of the brain. Rathke¹¹ first described an evagination from the oral ectoderm which enters into the formation of the hypophysis. This is very generally regarded as the anlage of the pituitary gland. It is known as Rathke's pocket. Rathke himself considered this pouch as the first step in the formation of the glandular portion of the hypophysis; its blind extremity finally comes into contact with the infundibulum and fuses with the processus infundibuli. A crescentic fold of oral mucosa subsequently surrounds the buccal orifice of the pouch and ultimately separates it from the mouth cavity. Luschka,¹² who agreed with Rathke, observed, in some human embryos, a small evagination from the mucous membrane of the mouth which was partly covered by a crescentic process of the oral mucosa. According to Dursy¹³ the cephalic end of the chorda dorsalis participates with the pocket of Rathke in the formation of the hypophysis. Maclay,¹⁴ in his studies on selachians, observed,

in many members of this class (particularly *Scymnus*, *Acanthias* and *Notidanus*), a connection between the mouth cavity and the hypophysis which persists throughout life. Landzert¹⁵ found what he considered a residuum of Rathke's pocket in new born infants.

The vigorous opposition of Reichert led Rathke¹⁸ to review his earlier work with the result that he changed from his original statement and decided that the oral evagination first described by him played no part in the development of the hypophysis. Wilhelm Müller⁵ was able to reaffirm Rathke's original conclusion that the anlage of the hypophysis is two-fold, the glandular portion arising from the ectoderm of the mouth, the neural portion from the processus infundibuli.

Many other embryologists have been of the same opinion, among them Kölliker²⁰, Dohrn,²¹ Froriep,²² Romiti,²³ Kraushaar,²⁴ Scott,²⁵ Balfour,²⁶ Emery,²⁷ Minot,²⁸ Hertwig²⁹ and Mihalkovics.³⁰ This view, however, was assailed in 1885 by Hoffmann,³¹ who was unable to verify the ectodermic origin of the pituitary gland in bony fish and reptiles. Kupffer³² likewise came to the conclusion that the gland did not develop exclusively from the oral ectoderm, and that an evagination from the primitive gut-tract entered into its formation. In amphibians Valenti³³ found the epithelial part of the hypophysis to be a complex structure, consisting of a secondary or accessory portion derived from the ectoderm and a principal portion from the entoderm. The ectodermic portion is to be regarded as the rudiment of some ancestral organ. According to Valenti, the diverticulum of the entoderm to which Kupffer attributes such importance atrophies and later disappears altogether, while a second evagination from the posterior wall of the fore-gut plays a greater part in the development of the hypophysis. The mode of origin of this latter diverticulum and the relation it bears to the branchial cavity are good reasons for considering the evagination as equivalent to the diverticula of the branchial fissures from which the thymus and thyroid bodies develop. Collina's³⁴ observations on the chick confirmed Valenti's findings. He does not entirely agree with Kupffer that the preoral entoderm contributes to the formation of the pituitary gland but is convinced that the

gland consists of a principal entodermic portion and a secondary ectodermic portion. Caselli³⁵ considers it impossible to accept the theory that the gland arises exclusively from the ectoderm. Prather¹⁰⁰ found, in *Amia calva*, that the glandular portion of the body springs exclusively from the entoderm. The anlage makes its appearance at the 160th hour of development, as a local differentiation of entodermic cells, immediately beneath the diencephalon. In its early stages the pituitary gland is a solid plug of cells. It first acquires a lumen about the ninth day from which time on a constantly increasing number of lumina appear. Balfour and Parker¹⁰² make the statement that, if it were not for the evidence that the pituitary gland is derived from the oral ectoderm in other types, they would be inclined to regard it of entodermic origin in *Lepidosteus*.

Phylogenesis

Müller⁵ is authority for the statement that there is no essential difference between the hypophysis of myxine and that of man. In Acraniates (*Amphioxus*) he could find neither pituitary gland nor infundibular process, because the elongation of the chorda beyond the end of the central nervous system prohibited the conditions necessary for the development of these structures. In Cyclostomes (*Myxine* and *Petromyzone*) the pituitary gland and infundibulum are both present, as is also the case in selachians. In the latter class, the glandular portion contains a small central lumen lined with stratified epithelium. A central vesicle was also observed in *Cyprinus carpio*. Among amphibians, the frog and salamander show a distinctive arrangement of the pituitary gland, having a superior portion in which the cells appear as masses and an inferior portion in which the cellular arrangement is aciniform. The infundibular process, in both instances, is rudimentary. In reptiles (*Testudo graeca* and *Emys picta*) the hypophysis is separated from the rest of the interbrain by the dura mater and here again it is possible to distinguish a superior and a inferior portion in the gland. The inferior portion contains a small central lumen lined with cylindrical epithelium. In the new born infant a similar cleft or lumen is found, which is undoubtedly the remains of the

original cavity in the pituitary anlage (Rathke's pocket). The adult human gland likewise presents a microscopic lumen or ventricle.

Andriezen³⁶ is of the opinion that from Müller on, investigators have assumed conditions in myxine to be primitive and so lost many good clues afforded by cephalochordates. *Ammocoetes* and the lower forms throw much light upon the function of the gland. From the lowest forms to man, the organ is morphologically and functionally two-fold. Investigations on larval *Amphioxus* show the existence of the so-called neural gland, in a position corresponding to the hypophysis. It has the histological characteristics of a secretory organ, is developed from the ectoderm and has connections with the floor of the brain and the roof of the mouth. Three elements enter into its formation.

1. A tubular aqueduct of capillary lumen, lined with ciliated epithelium, which establishes a direct communication between the mouth cavity and the thalamic ventricle. This he calls the bucco-neural duct.

2. A glandular body, the subneural gland, situated immediately above the buccal orifice of the duct.

3. A small group of nerve cells placed beneath the superior orifice of the duct.

This three-fold structure is found in *Amphioxus*, *Ammocoetes*, and *Balanoglossus* as well as in the adult and larval forms of *Ascidians*. Andriezen was able to prove experimentally the existence of an actual communication between the mouth cavity and the ventricles of the brain, which, he believes, provides a true water-vascular system for the central nervous tissues. To the collection of ganglionic cells at the upper end of the bucco-neural duct he attributes a function similar to that of the osphradial organ in mollusca, thus bringing it into general relation to the olfactory apparatus. It is his opinion that the hypophysis was functionally active in ancestral vertebrates, and that while the bucco-neural duct has become obliterated, the sub-neural gland and the collection of ganglionic cells have persisted.

Berkley,³⁷ by means of the silver method, studied the nerve elements in the hypophysis of dogs. He concluded that the organ has retained its double rôle of secretory and nervous function intact,

the former, perhaps modified, the latter, the original special sense organ, probably lying quiescent and only changed in so far as to admit of a slightly different arrangement of its elements. The comb-like figures and nerve tufts upon the central nerve endings resemble the terminations of the mitral cells of the olfactory bulb, the so-called olfactory glomeruli. They may accordingly be referred to some organ of special sense existing fully developed in the lower forms of animal life.

Development and divisions

Mihalkovics³⁰ describes four stages in the development of the hypophysis; (1) The formation of the hypophyseal angle; (2) the evagination of the oral ectoderm at this angle to form the hypophyseal pouch; (3) the separation of the pouch from the mouth cavity, its inclusion within the skull and fusion with the processus infundibuli; (4) the formation of the glandular acini. During this latter process the primitive hollow anlage of the pituitary gland is converted into a solid structure with the exception of a small cleft or ventricle situated close to the line of fusion with the processus infundibuli and representing the remains of the original pouch cavity.

Salzer³⁹ found that the anlage of the pituitary gland in mammals is a sac formed by a single layer of cylindrical epithelium which passes, without sharp demarcation, into the cuboidal epithelium of the mouth cavity. The further development depends upon the formation of the so-called pituitary acini, a process which affects the anterior and lateral walls of the sac but is not observed in the wall which is in contact with the processus infundibuli.

Gaupp³⁸ has shown that the anlage, in *Lacerta agilis* and *Anguis fragilis*, consists of a middle and two lateral evaginations from the oral ectoderm. The middle diverticulum becomes closed off from the mouth cavity and forms the main portion of the gland; the lateral evaginations are subsequently separated from the middle one and, perhaps, disappear altogether.

Chiarugi⁴⁰ states that these facts observed by Gaupp in the cases of reptiles, are equally true for mammals. Staderini⁴¹ found

that the pituitary gland in *Gongylus ocellatus* primarily consists of a single evagination but later presents a subdivision into a median and two lateral lobes. Rossi⁴² has shown that the lateral lobes in the chick are formed secondarily after the principal evagination from the oral cavity.

It is customary to regard the hypophysis as consisting of three parts, namely: (1) the anterior lobe representing the portion derived from the mouth cavity; (2) the posterior lobe derived from the interbrain; and (3) the stalk or stem connecting the posterior lobe to the infundibulum. These terms apply well to the human conditions, but cannot be used to describe the relation in many of the lower forms. Müller⁵ referred to the neural derivative as the processus infundibuli. This term is to be recommended for its general applicability, since it avoids the possible confusion arising out of the varying topography and relations to which this portion of the hypophysis is subject in different vertebrate forms. The derivative of the oral cavity, Müller called the epithelial portion. In this he distinguished two divisions or areas, the superior epithelial and inferior epithelial portions, a differentiation based, in part, on the topography and, in part, on the character of the cells composing the two portions.

Howell,⁶⁹ Herring,⁷⁰ Cleghorn,⁷¹ Cushing⁹⁸ and several others, have adopted a terminology according to which the hypophysis presents a posterior lobe, a pars intermedia, and an anterior lobe. The pars intermedia and anterior lobe are separated by a narrow cleft, the residual lumen or ventricle of the hypophysis. The pars intermedia belongs to the pituitary gland and is derived from the oral ectoderm. Gentes,⁴³ states that the glandular portion is constant throughout the vertebrate series but the neural lobe is not always present. The pituitary gland is formed of several segments which are never less than two in number. The segment in closest relation to the neural portion of the hypophysis, he calls the proximal or juxta-neural segment; the more remote portion he terms the distal segment. Because of the slight stainability in the juxta-neural segment, Sterzi¹⁰¹ calls this portion the chromophobic segment; the remaining, deeply staining, portion is the chromophilic segment.

The residual lumen or ventricle is a morphological feature of the hypophysis which has received much attention. Müller⁵ describes it as a narrow cleft in the pituitary gland along the line of contiguity between the anterior and posterior lobes. In transverse section it is U-shaped with the concavity toward the front. He observed the lumen in all animals investigated by him. Peremeschko⁵² states that the ventricle is to be found in all vertebrates, including the human adult. Its presence here has been denied, probably because it is not generally possible to obtain the body sufficiently early after death to prevent post mortem changes. Gemelli⁵⁶ regards the ventricle as an artifact produced by the fluids of fixation. Salzer³⁹ calls it the persistent remains of the saccular anlage of the pituitary gland. Trautmann,¹⁰⁷ in his work on domesticated animals (horse, pig, ass, bullock, sheep, dog, cat, etc.), observed a residual lumen in seven out of nine species examined, the horse and ass not presenting it. When present it was located between the pars intermedia and the anterior lobe. Löthringer,⁴⁶ believes that the colloid secretion of the pituitary gland is poured into this ventricle and absorbed from it by means of a dense vascular network. According to Haller,⁵⁶ there is a connection between the residual lumen and the subarachnoid space. This connection would bring the products of the gland's secretion directly into the cerebro-spinal fluid. Herring¹⁰³ is of the opinion that the colloid secretion derived from the pars intermedia, and perhaps, from the residual lumen, passes through the loose reticuloform tissue of the posterior lobe and so reaches the third ventricle, where it passes into the cerebro-spinal fluid.

Cells and cellular arrangement

Much confusion has arisen in the definition and description of the several types of cells in the pituitary gland. Flesch⁴⁴ first called attention to these different types, classifying them according to their affinities for different staining reagents. Some cells did not stain with acid stains, but had marked affinity for basic stains. These he called the chromophobes. A second type stain intensely with acid stains, these are the chromophiles. In the

text-books the terms are considerably confused, the chromophiles being spoken of as the chief cells in one instance and the chromophobes in another. Dostojewski⁴⁶ and Löthringer⁴⁶ confirmed the observations of Flesch. Rogowitsch⁴⁷ described a third type of cell which occurs in groups, the boundaries of which are so indefinite as to give them the appearance of the Langerhans cell masses observed in the pancreas. Rogowitsch regards the chromophobes as an indication of some pathological process, in which opinion Stieda⁴⁸ concurs. Schönemann⁴⁹ states that the chromophobes, whenever present, are to be considered pathological. St. Remy⁵⁰ demonstrated that the difference in staining qualities of the several types of cells was due to the presence of small granules in the cytoplasm. Benda⁵¹ agrees that, although there appear to be several types of cells in the gland, there are also many intermediate forms. He regards all of these cells as genetically identical, representing different phases of the gland's activity. Trautmann¹⁰⁷ distinguishes deeply staining chromophiles, faintly staining chromophiles, and chromophobic cells. Each of these types may be acidophilic or basophilic.

Concerning the arrangement of the cells in the pituitary gland, Guerrini⁹⁹ believes that the several types of cellular elements are not genetically related, nor do they represent different stages of functional activity. The two distinct types of cells are morphologically and functionally independent. The colloid material is not the only product of the gland. He describes a granular substance which, he maintains, is secreted by the granular cells of the organ. Peremeschko⁵² states that in some animals, particularly sheep, the cells are arranged to form definite acini which are grouped as more or less distinct lobules. Rogowitsch⁴⁷ describes three difficult areas in the gland of the rabbit; (1) the medullary layer, (2) the gland proper, and (3) the triangular space. The medullary layer consists of a peculiar type of cells which have a clear protoplasm. Numerous cysts of various sizes are found in this layer. The gland proper consists of acini whose true acinal character is often difficult to make out. The triangular space contains no acini or chromophiles. It is made up entirely of cell masses. Salzer,³⁹ comparing the arrangement in the pig and guinea-pig, found marked dissimilar-

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ities and concluded it was impossible to establish any type of arrangement that would conform to all species. Benda⁵¹ states that while the acinal character of the gland in man is evident in many areas, there are large fields in which it is impossible to discern any definite arrangement.

A rich vascularization in the glandular portion of the hypophysis has been recognized by all observers. Rogowitsch⁴⁷ speaks of the presence of a lymphatic system. Thaon,⁵³ in spite of repeated search, has never been able to discover any lymphatic elements in the gland. This author is not convinced that the colloid secretion is the only product of the gland's activity. Gentes⁵⁴ found that the hypophysis receives its blood supply from one of three arterioles which spring from a slender branch of the internal carotid artery. These arterioles enter the hypophysis by its inferior surface.

EXPERIMENTAL RESEARCH

Thyreoidectomy

A number of experimental methods have been employed to determine the functional significance of the hypophysis. Thyreoidectomy was among the earliest of these. Its aim was to establish a compensatory or vicarious activity on the part of the hypophysis in the absence of the thyroid gland. Rogowitsch⁴⁷ made a series of studies upon animals from which the thyroid had been removed. In dogs and rabbits he observed a constant hypertrophy of the hypophysis and for this reason believes that this organ and the thyroid gland are closely related in function, each of them, like the liver, contributing a substance to the blood which renders certain compounds innocuous, in the same manner that the internal hepatic secretion makes the ammoniacal salts harmless, or destroys the lactic acid and digestive ferments in the circulation. Hofmeister⁵⁷ confirmed these findings of Rogowitsch and Stieda⁴⁸ showed that, although thyreoidectomy always proves fatal to dogs, rabbits may survive the operation for many weeks. He agrees with Rogowitsch as to the compensatory hypertrophy of the hypophysis following complete removal of the thyroid. Leonhardt⁵⁸

obtained similar results from experimental thyroidectomy. In 112 cases of goitre Schönemann⁵⁹ found, in addition to the thyroid lesions, various types of pathological changes in the hypophysis characterized as follows: (1) a great increase in the number of chromophiles; (2) a great increase in connective tissue; (3) increased vascularization; and (4) increased formation of colloid material. These changes were disputed by Boyce and Beadles⁶⁰ but were later observed by Pisenta and Viola⁶¹ and also by Comte.⁶² Collina⁴ states that the compensatory or vicarious relation between organs of different functions is a general principle, indicating the retention of some element in their cells which persists in them as a result of their derivation from a common cell type. Moussu⁶³ demonstrated that rabbits die after complete thyroidectomy, though the hypophysis remains intact. Vassale and Sacchi⁶⁴ contend that if the hypophysis could compensate for the removal of so large a body as the thyroid, the thyroid should be able to substitute its activity after removal of the hypophysis. This is not the case, since all animals die after complete removal of the hypophysis, even though the thyroid remains intact. Schwartz⁶⁵ removed the thyroid from seventeen dogs, only two of which showed the changes in the hypophysis described by Rogowitsch. Blumreich and Jacoby⁶⁶ believe that the vicarious relation between the thyroid and hypophysis is not yet sufficiently well established.

Intravenous injection of extracts and pituitary feeding

The intravenous injection of pituitary extracts has yielded some interesting results. Cyon⁶⁷ observed that in dogs intravenous injection of the extracts caused an immediate decrease in the frequency of the heart beats; at the same time systole was energized and the blood pressure increased. Oliver and Schäfer,⁶⁸ employing extracts from the hypophysis, thyroid, and adrenal bodies, found that the most noticeable effect obtained from pituitary extracts was a marked rise in blood pressure. This rise was rapid and sustained according to the size of the dose employed. Thyroid extracts produced a distinct lowering of the pressure. In general, the action of pituitary extract more closely resembles the effects

produced by extracts of the adrenal body. Oliver and Schäfer do not agree that the thyroid and hypophysis are compensatory in function, since extracts produce such entirely different physiological effects. Howell's⁶⁹ conclusions are considerably at variance with this statement. He maintains that the effects of the glandular portion of the hypophysis alone are negative. The action resulting from extracts of the posterior or neural lobe are constant and well marked. With the vagi intact, the chief effect was a pronounced slowing of the heart together with an increase in blood pressure. Herring⁷⁰ found that extracts of the posterior lobe when perfused through the isolated ventricle of the frog's heart caused an acceleration and augmentation of its contraction. Cleghorn⁷¹ regards the hypophysis as consisting of two functionally distinct portions. Extracts of the pituitary gland produce little or no effect, while those obtained from the infundibular portion cause a marked retardation of the heart beat and a rise of blood pressure. Livon⁷² arrived at similar conclusions and adds that the effects of pituitary and thyroid extracts are in all respects identical. De Bonis¹⁰⁶ claims that the active principle of the posterior lobe is a substance very similar to adrenalin, while that from the anterior lobe does not resemble this at all. Sandri¹⁰⁶ believes that the pituitary gland contributes an antitoxic substance to the blood, which, under certain conditions, may become toxic. This latter phase explains the arrest of development in young animals (rats and guinea-pigs) under continued subcutaneous injections of the gland extract.

Some suggestive observations have been made with reference to the metabolic balance of nitrogen and phosphorus after pituitary feeding. Malcolm⁸⁷ found that the dried glandular portion, when given by mouth causes a slight retention of nitrogen, and that the neural portion produces a similar result. The entire hypophysis gives a directly opposite effect, with a marked increase in nitrogen output. Feeding of the dried pituitary causes a retention of phosphorus but increases the elimination of magnesium. According to Thompson and Johnston,⁸⁸ dried pituitary substance when fed to dogs, stimulates metabolism as evidenced by the increased nitrogenous compounds and phosphates in the urine. Loss of weight was also marked, although the animal, on the same

diet, had been gaining previous to the pituitary feeding. The effects do not immediately subside when this feeding is stopped. Gland substance obtained from young animals always gave the most pronounced results. Schiff⁷³ observed that the administration of the pituitary gland in the form of tablets to a young, robust man had no perceptible effects. In a case of acromegaly, however, this treatment produced a marked increase in the elimination of phosphorus pentoxide. The elimination of nitrogen when the pituitary extracts were administered showed that the increase of phosphorus pentoxide should not be attributed to a great decomposition of albumin but was more probably due to the decomposition of some tissue rich in phosphorus and poor in nitrogen, such perhaps, as the osseous tissue abnormally increased in acromegaly.

Hypophysectomy

One of the most interesting chapters of the problem of the hypophysis is based on the observation of hypophysectomized animals. Horsley⁷⁴ is credited with the first experimental operation of this kind. He completely removed the hypophysis from two dogs, which lived respectively five and six months, without manifesting any symptoms whatever. The animals were subsequently used for experimental stimulation of the cerebral cortex and in both cases showed a great increase in the excitability of the motor regions. Faradic currents of moderate strength produced a marked tetanus followed by severe, protracted epilepsy with clonic spasms fully as strong as seen in traumatic epilepsy in man. Gley⁷⁵ found that out of ten rabbits deprived of the hypophysis only one survived the operation. This animal had previously undergone a complete thyroidectomy. For three weeks following the removal of the hypophysis, the rabbit presented, at intervals, a series of slight convulsions, but survived the more remarkable trophic disturbances, such as thickening of the skin and marked asthenia, for a period of ten and a half months. Marinesco⁷⁶ removed the hypophysis from eight cats, two of which died of hemorrhage immediately after the operation, two died in twenty-four hours, two in five days and one survived for eighteen days. In the last

three cases he could find no evidence of infection or other definite cause of death. Vassale and Sacchi⁶⁶ removed the hypophysis from forty animals (cats and dogs). Out of fifteen dogs, one died of thrombus of the basilar artery, eight succumbed to infection 4, 7, 8, 13, 14, 17, and 18 days respectively after operation. Six died without signs of infection or any other complication 2, 3, 5, 6, 7, and 14 days after operation. Of fifteen cats, three died of infection in 3, 3, and 8 days, twelve died 1, 2, 2, 2, 3, 3, 4, 4, 4, and 8 days after operation without signs of infection or other complication. These authors are of the opinion that the complete removal of the hypophysis leads to death, prior to which the animal gives evidence of great psychical depression and marked change in disposition. In the later stages of the ensuing cachexia, the motor functions also present notable changes characterized by increasing fibrillary twitching, rigidity of the hind legs, unsteadiness of gait, appearance of tono-clonic spasms followed by slight attacks of dyspnoea. Anorexia alternating with polyphagia were constant symptoms. Vomiting, polydipsia, and polyuria were observed in nearly every case, and the temperature was usually below normal. In a later series of operations, one dog lived for thirty-seven days after complete hypophysectomy. The symptoms in this animal were at first similar to those already described but were considerably improved by the subcutaneous injection of pituitary extract.

Cyon⁶⁷ found that electrical stimulation of the exposed hypophysis caused changes in the heart beat and blood pressure, mechanical stimulation produced the same result but in a lesser degree. He believes that the hypophysis, because of its position in the most sheltered portion of the skull, its connection with the third ventricle, and its uncommon wealth in blood vessels, is especially fitted to be influenced by fluctuations of pressure in the blood and rachidean reservoirs of the skull cavities. When the hypophysis is removed, this mechanical safeguard is lost and the brain is subject to all the dangers of change in intra-cranial pressure. He agrees with Vassale and Sacchi that complete hypophysectomy in cats, dogs, and rabbits is always fatal. Gaglio,⁷⁷ on the other hand, working on frogs and turtles, states that death follow-

ing the removal of the hypophysis in mammals is due, in most cases, to direct complications of the operation. The majority of the frogs operated upon died in the course of a few weeks with symptoms of progressive paralysis. Autopsy showed a purulent basilar meningitis. One frog lived a month and a half, another, three months. Gaglio does not believe that these figures represent the actual resistance of the animals against the removal of the hypophysis, since the experiments were made in the summer, a season not favorable to frogs under laboratory conditions. With turtles his results were more satisfactory. Many of these died at the end of some days or weeks, but in all cases death was considered as due to some accident of the operation. Five turtles survived and continued to live in the garden of the laboratory under normal conditions and in apparently good health for several years. Subsequently some of them were employed for other purposes and showed a complete enucleation of the hypophysis. Gaglio concludes that some animals deprived of the hypophysis may survive a long time and remain in perfect health.

Narbout's⁷⁸ observations on nitrogen metabolism after removal of the hypophysis are noteworthy. He concludes that the organ has a definite function in the economy and is especially important to the growing animal. Damage to it leads to psychic depression, changes in the motor and sensory spheres, sometimes polyphagia and polydipsia. Removal of the gland causes increased elimination of phosphorus and nitrogen, lessens the interchange of gases and determines a great loss of weight, particularly in the nitrogen containing tissues. Friedmann and Maas⁷⁹ operated upon eighteen animals. Three of these were killed $2\frac{1}{2}$, 3, and 4 months after operation, showing but a slight remainder of the gland. Two died of infection, four of hemorrhage, and five, surviving for an average of fifteen days, showed no cause of death. One animal remained alive for three and one-half months and when killed showed that the hypophysis had been completely removed. They concluded that the hypophysis has no function in the adult animal. Friedmann⁸⁰ later reported a case of a young animal which survived the operation for nine months, remaining in this interval in a normal state of health. He believes that the organ is unimportant to the grow-

ing and adult animal alike. Casseli³⁵ thinks that the hypophysis is of much physiological importance. By means of its products and its relation to the nervous system, it maintains the equilibrium of certain toxic substances, whose predominance causes a rapid increase in certain tissues, but whose absence provokes a cachexia and arrests development. Livon¹⁰⁴ operated upon a dog which survived three months. Except for the few days immediately following operation, the animal showed no symptoms. At no time were any trophic or motor disturbances observed.

The customary procedure in removing the hypophysis has been by the oral or pharyngeal method as devised by Vassale and Sacchi.^{61b} Garnier and Thaon,⁸⁶ recognizing the dangers attendant upon this operation, employed what they term the temporal method (*voie temporale*) by which the hypophysis is approached through the temporal fossa. They operated upon twelve dogs, the majority of which died almost immediately after operation. One animal lived sixteen days, during which time it presented rapid emaciation, asthenia, feeble gait, anorexia, and a notable acceleration of respiration. Autopsy showed a marked laceration of the inferior temporal convolution and hemorrhage into the left cerebral peduncle. They conclude that the life of an animal deprived of the hypophysis may be prolonged for some time, but death is the ultimate outcome of the removal of the gland. Paulesco⁸⁶ also prefers the temporal operation. In fact, this is usually referred to as Paulesco's method. From his observations on twenty-four dogs and cats, he concludes that the hypophysis is an organ indispensable to life; the absence of it proves rapidly fatal.

Cushing,⁸⁸ in a large number of hypophysectomies on dogs, endeavored to overcome the so-called cachexia hypophyseo-priva by pituitary feeding, injection and transplantation. He aimed to prove the essential nature of the gland and also to observe the late results of partial removal. From this work he became convinced that total removal leads to a train of symptoms characteristic of no other known clinical condition. None of these symptoms occur after precisely the same operative procedure which stops short of actual removal of the gland. The conditions produced by total removal were so modified by gland injection and trans-

plantation as to prolong life beyond the usual post-operative limits, whereas the same modes of glandular therapy were effective in permanently sustaining animals which suffered from symptoms of a partial removal. In three cases the anterior lobe alone was removed, with the same results that attended total hypophysectomy. These cases lead him to believe that the anterior lobe is the most essential part of the gland, and is necessary to life. He agrees tentatively with Herring's idea that the acidophilic, hyaline bodies found in the processus infundibuli are actually secretory products en route to the third ventricle through the loose tissue of the neural lobe. Recognizing the bizarre nature of such an outlet of secretions, directly into the cerebro-spinal fluid, he thinks it may possibly represent a hitherto unobserved type of glandular activity. A state of adiposity is the most pronounced symptom after partial removal of the anterior lobe. This is accompanied by a secondary hypoplasia of the genital organs in adults or by a persistence of sexual infantilism if removal antedates adolescence. Polyuria, glycosuria, subnormal temperature, edema, hypertrichosis and psychic disturbance are among the more or less frequent occurrences after removal of the anterior lobe.

Gemelli⁹³ is of the opinion that the several different methods of hypophysectomy not only produce different post-operative symptoms but that the same operation in the hands of different operators likewise produces different results. The pharyngeal method of Vassale and Sacchi used upon dogs, cats, and rabbits gave a large variety of post-operative symptoms. Following Paulesco's method the motor disturbances were variable, while psychic disorders were more constant. Not infrequently the operation was immediately followed by some combination of the following symptoms: i.e., retarded respiration, anorexia, boulimia, polyuria, sometimes with sugar, sometimes with albumin; polydipsia and a depression of temperature. Gemelli concluded that this variability was due to some cause other than the mere ablation of the hypophysis. He, consequently, followed the identical operative procedures in other animals without disturbing the hypophysis. In these cases the same symptoms were observed. In view of this, he believes that the phenomena resulting from hypophysectomy, as described

by several investigators, are not characteristic of hypophyseal insufficiency. In a series of removals from frogs, the large majority died of infection. Six lived for an average of 106 days; five were killed at the end of four months; four at the end of a year, all showing complete enucleation. Four others were still living one and a half years after the operation. From these findings Gemelli concludes that hypophysectomy is not, in itself, fatal; that animals so operated upon do not present any immediate post-operative symptoms characteristic of the removal of the gland; that the hypophysis probably has not an indispensable function, and whatever its rôle, its influence plays a part only in development.

It appears, from a summary of experimental hypophysectomies, that ten investigators consider the organ indispensable to life; eight give evidence against its essential nature. In the latter group are Horsley,⁷⁴ Biedl,⁸⁹ Friedmann and Maas,⁷⁹ Lomonaco and Rynbeck,⁹⁰ Dalla Vedova,⁹¹ Fichera,⁹² Gemelli,⁹³ and Gaglio.⁷⁷ Those who believe it necessary to life are, Marinesco,⁷⁶ Vassale and Sacchi,⁸⁴ Gatta,⁹⁴ Caselli,⁸⁵ Pironne,⁹⁶ Paulesco,⁸⁶ Garnier and Thaon,⁸⁵ Livon,¹⁰⁴ Narbout,⁷⁸ and Cushing.⁹⁸

Sacerdotti⁸¹ has made a number of transplantations of the hypophysis in rabbits and white rats. In these experiments he made no cross grafts, but confined his transplantations to animals of the same species, the freshly removed hypophysis being imbedded in the subcutaneous tissue. In one case a small cyst was observed which contained a serous fluid and was lined with cylindrical epithelium. This, Sacerdotti thinks, may be interpreted as the remains of the bucco-neural duct. Guerrini⁹⁹ employed a variety of experimental methods to determine the effects of the hypophysis upon metabolism and trophism. Gravid animals showed a slight but constant increase of pituitary secretion. In the foetus at term or the new born animal the secretion appeared much less active than in the adult, but increased when the animal took the breast. Young and adult animals under such conditions as complete isolation, in temperatures above or below the normal, in acute and chronic anemias and profound inanition, gave evidence of changes in the functional activity of the gland. Particular significance is attached to the pituitary activity of animals in the various stages of starvation. In all of these cases a marked increase

of secretion was noted in the first few days; then followed a period of diminished secretion, while those dying of starvation showed scarcely more than a trace of the secretory products. Inoculations with certain exogenous toxins, such as diphtherotoxin and the blood serum of the electric eel, as well as some endogenous toxins as uric acid, xanthin, and biliary acids, seem to show an increased pituitary secretion as a direct result. Some animals suffering from auto-intoxication in consequence of ligation of the intestine, ureter, or common bile duct, gave evidence of a similar increase. Guerrini concludes that the hypophysis is functionally active in mammals and that such function as it may possess is manifested by the two types of secretion which it produces. These secretions do not, however, pertain to trophic conditions, but serve as general antitoxic agents to the biochemical processes of the organism.

CLINICAL AND PATHOLOGICAL OBSERVATIONS

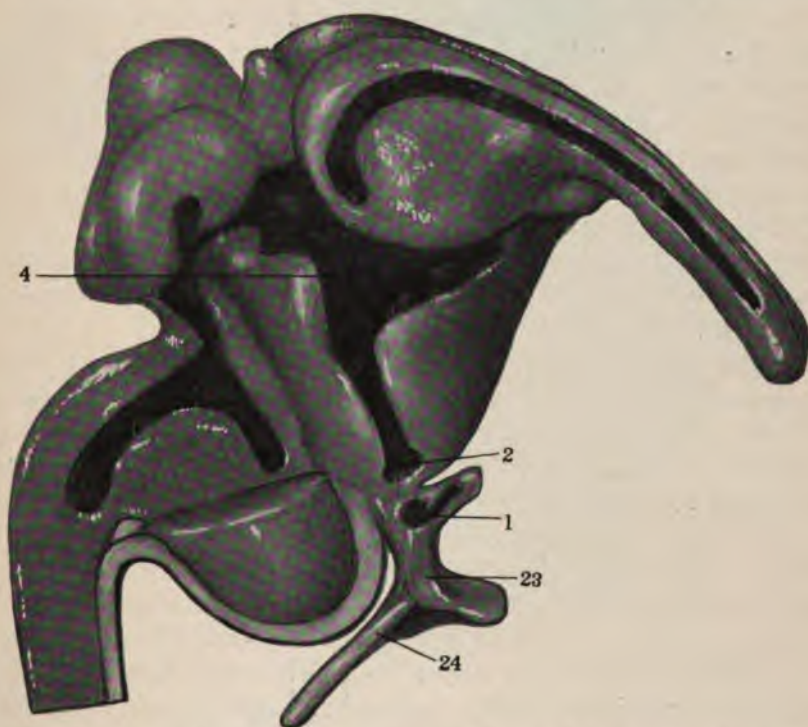
The controversy concerning the etiology of acromegaly and cretinism is still in the hypothetical stage. Acromegaly, (sometimes called Marie's disease) is considered by that author⁸² as a systematic dystrophy due to a perversion of pituitary function in which there occurs an accumulation of toxic substances in the extremities, producing an irritation of sufficient intensity to occasion the acromegalic alterations. Marinesco's¹¹¹ conclusions coincide with this view. Massalongo⁸³ thinks that acromegaly is due in part to an hypertrophy of the hypophysis, but also in part to the fact of the persistence of the thymus; both of these glands are looked upon as determining factors in the process of development. Their continued functioning during extrauterine life provokes the symptoms of acromegaly, as soon as the physiological period of development and growth have ceased in the body. Tamburini⁸⁴ describes two phases in acromegaly; in the first there is a functional hypertrophy of the gland with hypersecretion of those substances which tend to cause an increase in osseous tissue; the second phase is characterized by adenomatous or cystic degeneration with a cessation of glandular function which leads to cachexia and death. Collina⁴ regards acromegaly as due, not to a hyperactivity or perversion of pituitary function, but to an actual absence of all func-

tion. He draws attention to the almost analogous symptoms resulting from castration, which in the first place is characterized by an increase of the animal's body volume, and secondarily, by a depression of character. The absence of pituitary function manifests itself by changes which resemble those following removal of the sex glands in the male, while in both cases the nervous system plays an important rôle.

Arnold's¹⁰⁸ analysis of the sixty reported cases of acromegaly in the four years between 1890 and 1894 is particularly significant in this connection. He shows that the majority of cases on record up to that time with post mortem findings presented an enlargement of the hypophysis, which was generally a glioma involving the neural lobe. Intravital, this tumor gave rise to the typical symptoms of neoplasm in the hypophyseal region, namely, unilateral or bilateral temporal hemianopsia, optic atrophy, or total blindness with headache. Of the sixty tabulated cases of what must be classed as acromegaly, 61 per cent gave no symptoms of tumor in the region of the hypophysis; 35 per cent did have such symptoms, and 4 per cent showed enlargement of the gland at autopsy. This leads Arnold to state that "enlargement of the hypophysis is a more or less frequent manifestation in different stages of the disease." He is further inclined to recognize a distinct form of acromegaly, which he denotes the "pituitary type." He believes that many previous observers have laid too great emphasis upon the pathology of the hypophysis and bones, while they neglect the profound changes in the skin, muscles, thyroid, and the peripheral, central and sympathetic nervous systems. Thom's¹⁰⁹ observations contribute further evidence in this direction. He found that the hypophysis is subject to well marked changes during the course of certain acute and chronic diseases, among them being broncho-pneumonia, tubercular meningitis, acute leucemia, eclampsia, myocarditis, carcinoma and senile bronchitis. The principal changes were vacuolization, hypertrophy, hyperplasia, colloid degeneration, hyperemia and atrophy. He does not regard these changes in the hypophysis as causative in nature, but rather as specialized expressions in the pathology of the diseases in which they occur.

2. COMPARATIVE HISTOLOGY OF THE PITUITARY GLAND AND
A REPORT OF SIX HYPOPHYSECTOMIES

As a rule, two elements enter into the formation of the hypophysis, namely: (1) a prolongation from the floor of the third ventricle, the processus infundibuli and (2) an evagination from the mouth which extends upward until it meets the infundibular process. This pouch (Rathke's pocket) is derived from the oral ectoderm.



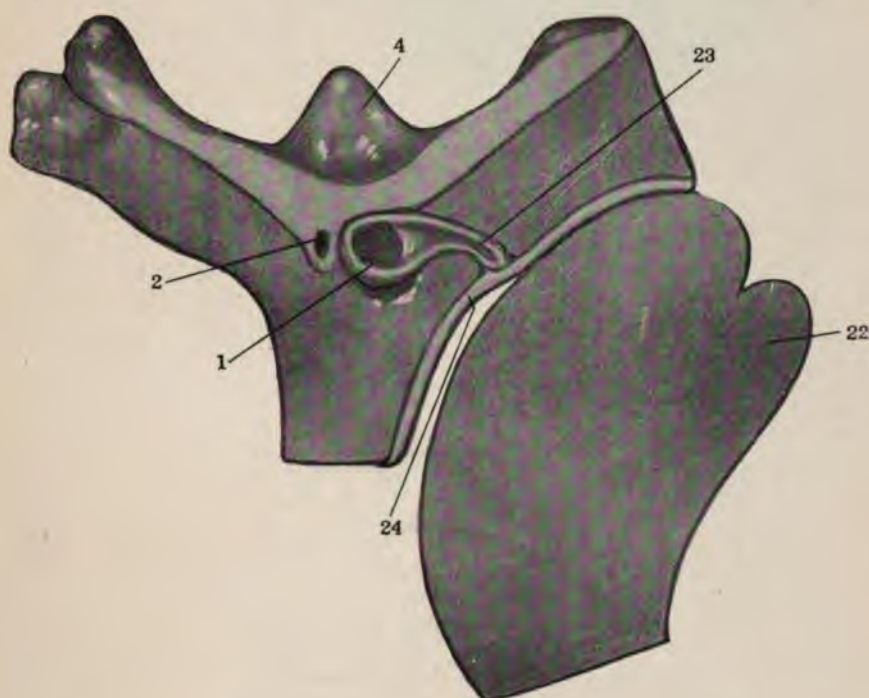
Text figure 1. From a reconstruction of 7.5 mm. *Scleroporus* embryo. 1. Rathke's Pocket. 2. Processus Infundibuli. 4. Diencephalon. 23. Pituitary stalk. 24. Oral mucous membrane.

It soon fuses with the infundibular process, thus forming a body situated beneath the brain and consisting of a neural and a glandular portion. These conditions are illustrated in text fig. 1, from a reconstruction of a 7.5 mm. embryo of *Scleroporus undulatus*.

The relative position of the two parts is subject to much variation in different species. In some instances, the oral pouch comes to lie in front of the infundibular process; it may, however, be located beneath or even behind the latter. As the two portions approach each other, the cells in the oral pouch begin to increase rapidly, especially along the surface of contact. When contiguity is accomplished, it not infrequently occurs, that a concave fossa is produced in Rathke's pocket in which the processus infundibuli rests. This juxta-neural surface later comes to form the juxta-neural epithelial portion, sometimes spoken of as the pars intermedia. The primitive relations referred to are seen in fig. 1, (Cat embryo, 6 mm.), fig. 2, (Chick embryo, 8 mm.) and fig. 3, (Aspidonectes embryo, 8 mm.). In *Aspidonectes* two accessory pouches arise from the main oral evagination. The importance of these and similar accessory pockets in the development of various species has been exaggerated, since it is a common tendency in many forms for the anlage of the gland to present multiple diverticula.

Rathke's pocket, at first in free communication with the mouth, soon gives evidence of a constricting process which rapidly reduces this connection to a tubular stalk (fig. 4). At this stage, the hypophysis is a large, infra-cerebral sac connected with the mouth by a slender stalk with a lumen. The stalk loses its lumen and the mouth is then in continuity with the sac by means of a solid stem. These conditions are illustrated in text fig. 2, from a reconstruction of a 12 mm. cat embryo. With the development of the primordial portions of the basisphenoid, the stem suffers obliteration, the hypophyseal sac loses all connection with the mouth and is included within the skull. It now forms an irregular, somewhat dilated vesicle in contact with the processus infundibuli. The surface of union with the infundibular process and the free surfaces behave differently in their further development. The cells in the free surface increase rapidly in number and cause a marked reduction in the size of the original cavity of the sac. This reduced cavity is the residual lumen. The cells in the surface of union do not proliferate as rapidly. At the time when this is taking place, the infundibular process is still a slender stem in contact

with the dorso-superior portion of the surface of union. As the latter surface increases, it tends to surround the processus infundibuli and, because of its intimate relation with this neural portion of the hypophysis, may be termed the juxta-neural epithelial portion. This portion continues to invest the infundibular process, carrying with it an extension of the residual lumen. Fig.



Text figure 2. From a reconstruction of a 12 mm. Cat embryo. 1. Rathke's Pocket. 2. Processus Infundibuli. 4. Diencephalon. 23. Pituitary stalk. 22. Tongue. 24. Oral mucous membrane.

7, (*Tupaia* embryo, 15 mm.) and fig. 8, (Cat fetus, 70 mm.) illustrate the conditions at this stage. In either case the infundibular process (2) is in contact with and almost surrounded by the juxta-neural epithelial portion (5). Anterior to this is a clear space of considerable size, the residual lumen or ventricle (7), whose ante-

rior wall is formed by the thick and rapidly growing distal epithelial portion (6). Among the lower vertebrates it was possible in all cases to recognize these two portions of the pituitary gland. The appearance and persistence of the residual lumen depend upon the mode of development and subsequent growth of the oral pouch. In *Rana* (fig. 5) the pituitary gland arises as a solid plug from the oral ectoderm; secondarily it acquired a lumen which disappeared in adult life. In *Amia calva* (fig. 6) the anlage of the gland is solid and only gains a lumen in the later stages. The juxta-neural and distal epithelial portions of all the lower forms present distinctive histological characteristics. The principal differences depend upon the staining qualities of the cells and also upon the cellular arrangement. In all species it is possible to recognize two varieties of cells. They will be classified here with reference to their stainability as basophiles and acidophiles, in order to avoid the confusion of the older nomenclature. Each variety presents two types: namely, deeply staining and faintly staining cells. The characteristics of the hypophysis listed below have been selected as a basis for the following histological study.

1. Infundibular Process (neural portion).
2. Saccus Vasculosus.
3. Juxta-neural Epithelial Portion.
4. Distal Epithelial Portion.
5. Cellular Elements:
 - (a). Basophiles { deeply staining.
 faintly staining.
 - (b). Acidophiles { deeply staining.
 faintly staining.
6. Cellular Arrangement and Topography.
7. Residual Lumen.
8. Blood Vessels.
9. Connective Tissue.
10. Colloid Material.

Petromyzon fluviatilis (figs. 9 and 10). In *Petromyzon* the pituitary gland is well marked; it presents certain of those characteristic features of which mention has just been made and thus falls definitely into place in the comparative series about to be studied. The gland lies beneath the prosencephalon, situated in a small

fossa of the cranial floor. With this portion of the brain it is in contact along its entire upper surface; its upper anterior extremity is in contiguity with a distinct processus infundibuli. The gland presents two well delimited parts, the juxta-neural epithelial portion and the distal epithelial portion. Each of these two divisions may be distinguished by clearly marked characteristics. The juxta-neural portion is the smaller of the two, its cells stain much more deeply with haematoxylin and at the same time are arranged in long, much convoluted cell cords. The distal portion, on the other hand, while it is considerably larger, does not exhibit the same disposition on the part of its cells to stain so deeply with haematoxylin, and, moreover, the cellular arrangement is entirely different, being that of long, thick cell-columns laid side by side and perpendicular to the floor of the skull. The other pituitary features in *Petromyzon* are entirely wanting; it presents no ventricle, no colloid material or connective tissue.

Squalus acanthias (figs. 11, 12, and 13). The hypophysis here also presents two well marked epithelial portions and a processus infundibuli. The latter process is situated anteriorly and thus reverses the usual relations existing between the epithelial and neural portions of the body. The tendency to form a long infundibular stalk was not observed; the neural portion of the hypophysis is a wide and thick walled diverticulum from the floor of the diencephalon. It comes into relation with the smaller of the two epithelial portions giving the latter its juxta-neural position. The distal epithelial portion is larger and more conspicuous. Influenced by the presence of an extensive saccus vasculosus, the two epithelial portions appear to adapt themselves to the floor of the diencephalon. This floor is marked by a sharp declivity beginning posteriorly at the mesencephalon, extending downward to a considerable length and finally turning into the horizontal as it gives contour to the infundibular process. With both of these planes determining the diencephalic floor, the epithelial part of the organ is in relation. The horizontal plane is in contact with the juxta-neural portion, is in fact fused with it. The oblique plane is in relation with the distal epithelial portion but not in contact with it, since a dense layer of highly vascular tissue is interposed between

them. It is obvious that the axes of the epithelial portions must meet in an obtuse angle and that the distal epithelial portion is situated dorso-superior to the juxta-neural portion. In outline the juxta-neural portion is triangular, with apex towards the chiasma and its long diameter extending antero-posteriorly. It appears as a blind sac whose floor and roof have been so closely approximated as to leave but a small residual space between them. The anterior two-thirds of the roof is fused with the infundibular process; the posterior third is fused with the distal epithelial portion. The floor of the sac is free except for contact with the meninges; the posterior wall is let out into several large irregular diverticula. The general contour of the distal epithelial portion is oval. Its inferior surface is concave from side to side, forming a fossa in which the juxta-neural portion, by the posterior third of its roof, is lodged. The arching process by which the distal portion adapts itself to the juxta-neural portion carries the cells of the former over the roof of the latter and then for a short distance into the floor.

The saccus vasculosus is composed of a rich plexus of wide capillary channels extending caudad from the diencephalon; it contains a cavity which is in direct communication with the third ventricle and lined by an ependymal prolongation from that chamber. Continuing its course dorsalward for a short distance the sac describes a sharp ventral curve and sweeps forward until it comes in contact with the posterior surface of the distal epithelial portion of the gland, undergoing fusion with the latter along the line of contact. The saccus vasculosus forms a diverticulum from the diencephalon having a distinct lining throughout, which, however, varies somewhat in different areas. Along the anterior wall it is of irregular thickness, in some places giving the appearance of an actual invasion into the neural substance and being a number of cells deep (5-6). The same is true of the more anterior parts of the superior wall. Along the inferior wall (the area corresponding to the superior surface of the gland) the membrane is very much attenuated, seldom showing a depth of more than one or two cells. Some of the serial sections from this piece, clearly demonstrate the communication between this infundibulo-saccal cavity and the

ventricle of the diencephalon. The presence in this cavity of a peculiar homogeneous substance which stains deeply with eosin is also noteworthy. Although each of the several ventricular cavities contains a lightly staining material which probably pertains to the choroid plexuses, the substance in the cavity of the infundibulum is to be differentiated by its more intense eosinophilic properties. It resembles, in its general appearance, the colloid material found in the pituitary gland of many other animals and its proximity to the dense glandular columns of the gland in this particular case, as well as the thin partition wall separating the hypophysis and the infundibulum serve to suggest that the gland's secretion is thrown directly into the infundibular cavity, or finds its way to this destination as an elaboration of the saccus vasculosus. The juxta-neural portion of the gland presents itself as a slender lobe extending forward along the pars optica in the direction of the optic chiasma. Its anterior extremity is sharply pointed, while through its entire length is seen a long narrow cleft or cavity, the residual lumen. This tongue-like process is conspicuous only in and about the median planes, presenting, in sagittal section, the general contour of a triangle whose apex points toward the chiasma. The superior wall of the juxta-neural portion is relatively thin and has a smooth internal surface; the inferior is much thicker and thrown into numerous villiform processes which encroach upon the central lumen. Many small vesicles appear in the upper and lower walls, but the residual lumen itself ends blindly and has no communication with the chamber of the infundibulum. Unlike this latter cavity, the lumen gives no evidence of any contained substance whatever. Distinctive as are these features in the two glandular portions of the hypophysis, a still greater contrast is drawn between them by their cellular differences. The distal epithelial portion is made up exclusively of acidophilic cells whose cytoplasm is scanty. These acidophile cells are arranged in long parallel columns whose general direction is perpendicular to the base of the skull. Only a few faintly staining acidophiles were observed. In the juxta-neural portion, on the other hand, (especially in the villiform processes of the inferior wall) the epithelial cells are all much larger, having oval nuclei

and a relatively extensive cytoplasm. In form they are cuboidal or low columnar; their arrangement is by no means as discrete as that observed in the distal portion, in fact the general disposition of these cells is most irregular, except in a few isolated places where there is seen a distinct tendency on the part of the cellular elements to arrange themselves in acinal formation. This tendency is noticed not only in the villiform processes but also in the neighborhood of the small diverticula already mentioned. The characteristic feature of these cells, however, is their apparent aversion for acid and their marked affinity for basic stains. In another point the two epithelial portions differ, that is, in their respective vascularization; the distal portion has a greater wealth of vascular elements which are arranged in special relation to the columns of acidophilic cells so that each long cell column has a corresponding long capillary column on either side of it. It is possible that this condition is of significance as to the functional activity of the gland, whose distal portion, it will be remembered, is composed solely of eosinophilic cells, while its blood spaces drain into the plexuses of the saccus vasculosus. *Squalus acanthias*, therefore, represents a type in which not only are the two cell varieties kept distinctly apart but each has its own distinctive arrangement.

Anguilla vulgaris (figs. 14, 15, and 16). The hypophysis of the common eel is, in several respects, particularly suggestive. It affords another example of the complete segregation of the different cell types into distinct regions. Still more striking are the unusual relations and conditions observed in the processus infundibuli and the juxta-neural portion. A modeled reconstruction made from serial sections shows the hypophysis to be of irregular, pyramidal shape, with two broad and flattened surfaces, one presenting toward the base of the diencephalon, the other toward the floor of the skull. The base is directed posteriorly and upon analysis is found to consist of the neural and the juxta-neural portions. The rest of the body, as it tapers toward the anteriorly directed apex, is more complex in its make-up. Springing forward from the anterior aspect of the juxta-neural portion, about the median line, is a slender shaft of cells, which extends a considerable distance toward the front, gradually growing thicker as it approaches

its anterior extremity, where it spreads out very much like the head of a mushroom. Because of its position, this region may be designated the area centralis. Surrounding the stalk of the area centralis on its lateral and ventral aspects is another mass of cells which stands out in strong contrast due to the staining properties of its cells. The bulk of this cell mass is lateral of the stalk and may be termed the area lateralis. This area, therefore, forms a partial collar about the central stalk, leaving it uninvested upon its dorsal or cephalic surface. It reaches forward so as to become lodged in the concavity of the mushroom-like head of the area centralis. The area centralis with the area lateralis constitutes the distal epithelial portion of the gland.

The infundibular process is well marked and projects backward from the floor of the third ventricle with which it is in free communication. Its stalk is slender and has a wide lumen. After extending backward for some distance, this stalk begins to arborize, giving off primary, secondary and tertiary branches which extend in all directions. Each branch has its own lumen, which establishes ultimate connection with the main lumen of the stalk and thus with the third ventricle. Meshed in between the branches of the stalk are masses of well defined cells which make the whole structure appear as a branched tubular gland draining into the encephalic chambers. Closer study shows the neural character of the stalk-tissue, while its lumen is lined throughout by typical ependyma, even to its finest branches. Moreover, the relation of the epithelial-like cells can scarcely be other than that of mere juxtaposition. Yet one can hardly avoid the conclusion that here is a glandular structure, at least potentially equipped, with all the necessary elements, including acini, interlobular ducts and common duct, to contribute a secretory product to the cerebro-spinal fluid. Such a view becomes still more significant in the light of Herring's contention that the hyaline masses found scattered through the neural portion of the hypophysis, in some mammals, are products of secretion en route to the third ventricle by way of the loose tissue of the processus infundibuli. However the facts may stand, it seems clear, on morphological grounds, that the much branched diverticulum from the floor of the third ventricle is the neural por-

tion of the hypophysis, while the epithelial cells, investing it so intimately, constitute the juxta-neural epithelial portion. The cells of this portion are exclusively of the faintly staining basophilic type and are arranged as irregular masses. In size, they are relatively small, their nuclei are picnotic, their cytoplasm clear and their boundaries not very definite. Each of the two regions of the distal epithelial portion contains a specific variety of cells. In the area centralis the cells are exclusively of the deeply staining basophilic type. Their nuclei are large and oval, the cytoplasm fairly abundant and their boundaries clear. They are arranged as irregular masses, situated among which are a great number of acini-form clusters. The cells of the area lateralis are exclusively of the deeply staining acidophile type, their nuclei are large and reticular, and their cytoplasm scanty. They are arranged in thick columns, intervening between which are large blood spaces. In the region in which the stalk of the neural portion enters the gland, the cells of the area centralis form a small bridge passing over into the juxta-neural portion. Nothing that could be considered a residual lumen was found. For specification of other details see appended tabulation.

Lepidosteus osseus (figs. 17 and 18). With some modifications, the conditions observed in the eel repeat themselves in the gar-pike. The divisions of the body, although present, are not so much accentuated, while the complete investment of the reticular infundibular process by the juxta-neural portion, which is the dominant feature in the *Anguilla*, is less clearly drawn in *Lepidosteus*. The body lies immediately beneath a spacious infundibulum, from the floor of which a short infundibular process projects downward, carrying with it a narrow lumen. This process quickly enters the juxta-neural portion and there breaks up into a number of branches, each branch having its own lumen. The epithelial cells which surround this reticular infundubular process are of the faintly staining basophilic type. Anterior to the point at which the process enters the gland there is a narrow zone of basophiles, arranged in a dense stratum, very similar in all particulars to the juxta-neural eipthelial portion in *Rana*. These basophilic cells have a larger cytoplasm and smaller nuclei than the cells investing

the processus infundibuli; at the same time they contain fine granules in their protoplasm. Whether there is any justification for, considering these two cell varieties as distinct types is a question, yet it may well be that the basophiles in immediate relation to the infundibular process correspond to those of like topography in the eel, while the massed basophiles situated anteriorly may represent the basophilic area centralis found in *Anguilla*. In front of this mass of basophiles lies a larger area containing nothing but acidophiles, so that the basophiles and acidophiles in the pike are also distinctly separated. The acidophiles are smaller elements, their boundaries are less definite and their cytoplasm clear. They are arranged in thick columns, between which are irregular blood spaces which drain into a series of channels placed between the gland and the floor of the interbrain. These channels in turn come into relation with a large sacculus vasculosus posterior to the juxta-neural portion and in free communication with the third ventricle.

Diemyctelus viridescens (figs. 19 and 20). The hypophysis of this animal rests upon the flat base of the skull, immediately beneath the cephalic end of the mesencephalon. Its position and relations are both somewhat unusual, for it gives the impression of having been forced backward by the processus infundibuli, so that this latter structure, instead of occupying its customary position behind and somewhat above the pituitary gland, here lies in front of it. In the median plane the gland, by its anterior wall, is in close contact with the posterior infundibular wall, although an appreciable cleft or space extends from top to bottom, along the line of contact. The posterior wall of this cleft is formed by the large columnar epithelial cells; the anterior presents a thin, nucleated membrane, abutting against which is a chain of widely distended capillary vessels. The sections through the more lateral planes indicate that the glandular body is not in contact with the processus infundibuli, but is surrounded by a rich, capillary network. Unlike the hypophysis of *Squalus acanthias*, the epithelial portion presents no accessory division but exists as a single lobe. The juxta-neural portion is present as an irregular layer of deeply staining basophiles. The cellular arrangement in the distal epithelial portion

is such that the cells seem to run in long cords antero-posteriorly and parallel to the base of the skull. An occasional acinus was also observed. Between the cell cords are long capillary channels which give the organ a highly vascular appearance. The cells of this portion are all of one type, being large cuboidal elements, with large centric nuclei surrounded by a more or less granular protoplasm. They show marked affinity for acid stains. Their boundaries are clear, and their entire conformation associates them with the acidophilic cells already mentioned in the gland of other animals. The glandular structure affords absolutely no evidence of the basophilic cells. The residual lumen is also exceptional because of its unusual position, lying as it does, along the anterior wall of the gland. In the majority of instances it is found along the posterior wall. The lining of this ventricle, as previously stated, partakes of the nature of a syncytium over the anterior wall; the posterior wall has a distinct layer of high columnar cells. At some points this ventricle is so narrow as to be negligible but in other places it is wide and conspicuous. The epithelial elements of the gland receive their support exclusively from the blood vessels, which course between the cell cords. No colloid or other material appears in the ventricle.

Menobranchus lateralis (fig. 21). The hypophysis of this animal is very similar to that of *Diemyctelus*. It consists of a wide, thin walled infundibular process which approaches the gland portion from the front, fuses with it along the line of contact and is invested by a typical basophilic juxta-neural portion. The gland thus contains two types of cells, the deeply staining acidophiles and basophiles. The juxta-neural portion is very irregular in depth but is otherwise typical. No residual lumen was observed and no connective tissue elements other than those concerned in the blood vessels were found.

Rana sylvatica (figs. 22 and 23). In *Rana sylvatica* the hypophysis presents the two distinctive divisions, namely, that portion derived from the ectoderm of the oral cavity, the pituitary gland, and the portion derived from the neural ectoderm, the processus infundibuli. The body is situated in a shallow depression in the base of the skull. In sagittal sections, it has the appearance of a

right angled triangle whose base is parallel to the skull floor; its altitude is free except for the arachnoid membrane, while its hypotenuse is contiguous with the thin floor of the infundibulum; a large area above the superior angle is in contact with the processus infundibuli. This infundibular process, in comparison with the rest of the body, is relatively small. The pituitary gland itself presents a juxta-neural and a distal epithelial portion, the latter of which is the more conspicuous, both because of its greater size and the characteristic arrangement of its cells. The cells of the distal portion are exclusively of the acidophilic type; their nuclei vary decidedly in form from the small spherical to the large ovoid elements, both of which are very granular and stain deeply with the basic stains. The protoplasm of these cells is relatively scanty; it contains but few granules, while the cellular boundaries are fairly well defined. These cells are arranged in columns running perpendicular to the base of the skull and separated from each other by very considerable intervals, in which are observed fine chains of capillaries. The juxta-neural portion is smaller; it seems to have no affinity for acid stains, but its cells react to haematoxylin and hence are basophilic. Their nuclei are uniform in size and shape, being large, granular and spherical. Their cytoplasm contains no granules, is greater in amount than in the case of the acidophilic elements and the cellular limits are more clearly cut. The important observation here is, that the pituitary gland in the frog is composed of two types of cells, the acidophiles and the basophiles; each cell type is distinctly segregated in its own particular area of the gland, so that there is not the slightest intermingling of the two varieties. The cells in the juxta-neural portion present no distinctive arrangement; they are massed together apparently with total lack of design, although they are not so densely crowded as the neighboring cells in the distal portion. While these cellular differences give marked distinguishing characteristics to the two portions of the gland, the latter are to be differentiated in still another particular, namely, their blood supply; in the juxta-neural portion there is a conspicuous absence of all blood-vascular elements. This is in strong contrast to the distal portion where the vascularization is rich and shows the characteristic intercortical

arrangement. In the larval stages, the hypophysis has a residual lumen or ventricle; it is observed at the junction of the infundibular process with the juxta-neural portion and presents itself as a relatively wide cavity whose upper and lateral walls are formed by a thin syncytial membrane while its inferior boundary is represented by the peripheral epithelial cells of the juxta-neural portion of the gland. This cavity disappears entirely in the adult stage. A careful study of a large number of sections revealed no evidence of any substance in the ventricle. The presence of any connective tissue framework was sought in vain, although this office seems to be amply filled in the distal portion by the chains of capillary vessels.

Cistuda carolina (figs. 24 and 25). The hypophysis of this animal is found in the deep fossa of the sella turcica. At first sight, its general microscopic appearance indicates a structure by no means as easy of interpretation as those of the lower forms already discussed. It presents a saccus vasculosus, a processus infundibuli and a pituitary gland. The infundibular process extends backward and slightly downward from the infundibulum to come in contact with the upper and posterior surfaces of the pituitary gland, while the main body of the latter extends forward in the direction of the chiasma, its upper surface lying beneath the floor of the infundibulum. Both the juxta-neural and distal epithelial portions are present, the former placed along the upper surface of the gland, lining the residual lumen and composed exclusively of faintly staining basophiles. To some extent it also extends backward over the posterior surface of the gland where it interposes itself between the saccus vasculosus and the distal epithelial portion. Its general arrangement is that of an irregular stratum. This irregularity is particularly evident along the line of fusion with the infundibular process, where the cells appear to be collected into a dense mass or stretched out as an attenuated layer adherent to the processus. The distal portion constitutes the major part of the gland and is composed exclusively of the deeply and faintly staining acidophiles of which the former are more numerous. An accessory, tongue-like projection extends forward from the anterior, inferior angle toward the chiasma.

In the instances already cited the distribution and arrangement of the cells in the epithelial portions of the body have served as one of the chief particulars of differentiation between the two divisions of the gland. Here, however, the tendency of the two types of cells to separate themselves into discrete areas seems less well defined, for, although both the basophilic and acidophilic cells are present, they are more closely associated and give the glandular structure a more complex appearance than in the lower forms. The great majority of the cells in the gland are acidophilic, the deeply staining elements preponderating. These cells affect the more central areas of the distal portion. Spreading out to the peripheral regions they become perceptibly reduced in number and are entirely replaced along the cortex of the gland by the larger basophiles. It is a prominent characteristic of these basophilic cells, in the species thus far examined, that their nuclei are uniformly of the same size and always spherical, while the protoplasm is clear and considerably more extensive than in the case of the acidophilic cells. The acidophiles, on the other hand, present nuclei which vary both in conformation and size, while they occupy, as a rule, an eccentric position in the cytoplasm. It is the arrangement of its cellular elements, however, that distinguishes the pituitary gland of the turtle from the lower forms and gives it an apparently more complex appearance. Instead of the simple columnar formation, it presents what seems to be a distinctly acinal arrangement. In the case of the turtle, both in transverse and sagittal sections, the cell cords have given place to a formation which at first glance seems to indicate the presence of actual acini. It is far more probable that, instead of a metamorphosis from the original cell-cord arrangement to actual acini, the prototype still persists, in such a modified form, as might well be the result of a simple process of convolution. This view is in part substantiated by the presence in the gland, especially along its peripheral borders, of long, unconvoluted cell cords, identical in all respects to the cords observed in the lower forms. In the central areas the general law for increasing epithelial surfaces by simple reduplication seems to have been followed and the cell cords are thrown into convolutions. A further peculiarity of the gland in this animal is the

occurrence of masses of basophilic cells, which are interpolated between the convolutions, but entirely independent of them. These cell masses are seen only at or near the centre of the distal portion of the gland. For the most part, the convolutions are solid and of very irregular dimensions; in some instances they present distinct lumina containing a homogeneous substance which stains deeply with eosin. A delicate, but pronounced network of connective tissue ramifies between the convolutions while the blood vessels, entering posteriorly from the saccus vasculosus, distribute themselves irregularly and apparently without regard to the intercordal arrangement heretofore observed. A striking feature of the gland is its residual lumen or ventricle. It is a cavity of considerable size, situated between the upper surface of the superior portion of the gland and the floor of the infundibulum with which latter it is parallel. Its lining differs in several particulars from those already described, inasmuch as it seems to be enclosed by a thick epithelial wall, especially in the sagittal plane, although this layer appears to undergo an attenuation as it extends outward from the median line; in the lateral areas, it has been reduced to a thin, membranous lining. The cells in the thicker portion of this wall are of the basophilic type and are arranged both above and below in a stratum several layers deep. The anterior wall, although very short, is much thicker than the superior, while posteriorly the cavity dips downward for a short distance into the more compact areas of the distal portion. All of the basophilic cells surrounding this residual lumen are so placed as to be perpendicular to the long axis of the cavity. No colloid or other material was observed in this chamber, and the only evidence of any such substance was found in those cell cords which presented lumina.

Eutaenia sirtalis (fig. 26). The hypophysis of the garter snake presents an infundibular process, a pituitary gland and an extensive saccus vasculosus. The infundibular process lies in front of the gland and is itself a structure derived from the floor of the infundibulum. The saccus vasculosus is situated behind the gland and surrounds it on all sides, while the gland, thus embedded laterally and posteriorly, is contiguous with the processus infundibuli

in front. Two portions of the gland may be recognized, the juxta-neural and distal epithelial portions. The juxta-neural epithelial portion invests the infundibular process as a thin stratum of faintly staining basophilic cells, one to three cells deep. It fails to cover the inferior surface of the processus infundibuli but as it sweeps over the superior surface it becomes greatly thickened to form an irregular mass of cells. It also forms the anterior wall of the residual lumen. The distal epithelial portion comprises faintly staining acidophiles; the cells are larger than the basophiles, their nuclei distinctly granular and their boundaries clearly marked. The general tendency of these cells is to arrange themselves in long cell cords and, occasionally, apparent acini. The cells of the two portions of the gland are thus completely separated. A typical residual lumen is seen at the junction of the gland and the infundibular process. Its anterior wall is composed of a stratum of basophilic cells. The posterior wall is irregular and blends with the distal epithelial portion. No blood vessels were observed in any part of the gland, the vascular elements being processes of the saccus vasculosus which penetrated the gland tissue. No colloid material and no connective tissue elements were observed.

Alligator mississippiensis (figs. 27 and 28). The hypophysis consists of an infundibular process, a juxta-neural and a distal epithelial portion. The infundibular process projects backward and downward from the infundibulum carrying with it a wide cavity. It lies above the glandular portion. The process itself becomes thickened at its posterior extremity and into this thickening the cavity sends out short irregular arms. This invasion of the neuroid tissue of the processus by branches from the main infundibular chamber resembles the conditions already cited in *Lepidosteus* and *Anguilla*. Moreover, the entire, thickened extremity of the infundibular process has been rendered porous by the presence of numerous spaces, each having a distinct lining suggestive of ependyma. In one instance, at least, such a space was traced to a direct connection with the infundibular cavity and received its lining as a continuation of the ependyma. The juxta-neural epithelial portion almost completely invests the processus infundibuli, covering its anterior, posterior and inferior surfaces. In its

entire extent, it forms one of the walls of the residual lumen. Its cells are of the faintly staining basophilic type and are arranged in a stratum of irregular thickness. The distal epithelial portion is oval in shape and lies beneath the residual lumen. Both types of cell are present, the acidophiles tending to occupy the central areas of the gland, while the basophiles form a narrow zone along the periphery. The stainability of both varieties of cell is less active than in the other tissue already examined, for which reason the cells must be classified as the faintly staining acidophiles and basophiles. In arrangement, both types present apparent acini and cell cords. Some of the acini have lumina and a few contain colloid substance. From the supero-anterior extremity of the gland, a long accessory process projects forward toward the chiasma. A saccus vasculosus is situated above and behind the body but in much less intimate relation than in *Eutaenia sirtalis* or *Cistuda*. Blood spaces, in a rich plexus, ramify along the distal epithelial portion, while a delicate framework of connective tissue supports the gland.

Gallus domesticus (figs. 29, 30 and 31). The hypophysis of the common fowl presents a pituitary gland and an infundibular process. The gland lies beneath the infundibulum and the infundibular process. It is fused with the entire inferior surface of the processus infundibuli and with a part of the same surface of the infundibulum itself. This continuity of structure applies, however, to but a small portion of the glandular tissue; between the main epithelial division and the infundibular region there intervenes an appreciable space or cavity, the residual lumen or ventricle. The juxta-neural epithelial portion, though present, is not well developed, being but a thin layer of basophiles almost completely surrounding the processus infundibuli. The distal epithelial portion is conspicuous in that it comprises both types of cell, i.e., basophiles and acidophiles, but each confined to its own particular area. The basophiles are of the faintly staining variety. They form a thick stratum bounding the residual lumen anteriorly, and are arranged in mass formation. An occasional deeply staining basophile is found scattered in this layer. A narrow zone of basophiles surrounds a central area comprised exclusively of both varieties of

acidophiles. The central areas of the glandular tissue differ in certain particulars from the peripheral areas, while both of these in turn are to be distinguished from the region immediately surrounding the residual lumen, the circum-ventricular area. It must be admitted that the several points of distinction depend upon slight variations of structural detail. Their aggregate differences, however, are sufficient to produce a well marked regional differentiation. The central area is characterized by a profuse interspersation of deeply staining acidophiles among a greater number of faintly staining acidophiles, in such a manner that the former elements have the appearance of being embedded in an extensive background of the latter. In the central area the acidophilic cells display a tendency toward aciniform arrangement. In some instances the lumina of these apparent acini contain a homogeneous substance which has a marked affinity for acid stains. This aciniform arrangement of the acidophiles is not the only form in which these elements appear in the central area; in some places there are long chains or cords of cells taking an irregular course and standing out against a background of the more faintly staining acidophiles. Three types of cells may be recognized in the central area of the gland: (1) small, irregular cellular elements with scanty cytoplasm and a large picnotic nucleus, situated somewhat eccentrically and staining deeply. The protoplasm takes the acid stains to a slight degree. These are the cells which form the apparent acini and cell cords. They stand out in relief against the ground work of faintly staining elements. Due, in great measure, to their dense crowding the cell boundaries are difficult to make out. Their nuclei lie close to the lumina of the acini. (2) The second type of cell presents a large, granular protoplasm containing a granular nucleus situated near the center. The cytoplasm stains faintly with acid stains. In their arrangement these cells show no definite design but are scattered throughout the central area as large, irregular masses and cords. Their general characteristics and staining properties bring them into the category of acidophilic cells. (3) The third type of cell in the central zone is the very large and isolated acidophiles whose cytoplasm as well as the nucleus stains deeply with eosin and whose dimensions, in comparison

with the other cells of the gland, are strikingly large. These cells are found in no other region and even here are rare. The peripheral area is a narrow zone lying outside of the central area and along the free border of the gland. It is characterized by the presence of the typical aciniform arrangement of the basophilic cells which, in this case, do not stand out as prominently as in the central area, for the reason that their background is no longer composed of the large acidophilic elements but is entirely made up of large basophiles. These latter cells are of the faintly staining variety. A few deeply staining basophiles are found in the peripheral area. However decisive this demarcation between the peripheral and central zones may be, it is far less pronounced than the difference between the central and circumventricular areas. The chief feature in this transition is the sudden disappearance of the deeply staining acidophiles which are so prominent in the other areas and whose typical arrangement is aciniform. Almost as if stopping at a barrier, these cells disappear from the area immediately about the cleft of the residual lumen; such cells as do occur there have lost all tendency to the characteristic acinal formation and are massed as a deep epithelial stratum just beneath the ventricle. As a rule these cells in the circumventricular area are large, with a clear protoplasm and a granular nucleus; they have a mild affinity for basic stains and belong to the faintly staining basophilic variety. Not only do these cells and this arrangement characterize the circumventricular zone along the upper and lower wall of the residual lumen but the same structural features are observed in two epithelial protuberances which project laterally from either side of the gland and may be called the lateral lobes. The epithelial stratum forming the upper limiting wall of the residual lumen is much thinner than that of the lower; in all other respects it is similar to it. Projecting backward and somewhat upward so as to almost encapsulate the infundibular process, a slender extension makes its way from either lateral process; it partakes partly of the characteristics of the circumventricular area and partly of the peripheral zone; at the sametime it presents certain features distinctly peculiar to itself. These two dorso-lateral projections of the glandular structure still retain a narrow prolongation of the

residual lumen, and, although they apply themselves to all the surfaces of the processus infundibuli, there remains a small strip of the latter, posteriorly and in the median line, which is not thus covered. Interpreted in the light of embryological knowledge, it seems that in the course of its development the infundibular process first came into contact with the posterior surface of Rathke's pocket and subsequently produced an invagination in this surface. Lodged in this depression, the processus was almost entirely surrounded by the epithelial tissue of the posterior wall. It is with this wall that fusion finally takes place between the infundibular process and the pituitary gland. Throughout its entire extent the wall shows no variations, being a thin stratum of cells with an affinity for basic stains alone. The other limiting wall of the residual lumen is likewise made up of basophilic cells; these are not arranged as cell masses, but show a distinct tendency to form typical cell cords and acini, some with and some without lumina. The dorso-lateral protuberances contain many large and small blood spaces which distinguish these two areas from all other zones of the gland. In the center of the apparent acini, as well as in the lumina of the cell cords, large clumps of some homogeneous substance staining with haematoxylin were observed; they vary in size and shape, being ovoid or circular in outline, depending upon whether they are seen in the lumen of a cell cord or in that of an apparent acinus. They constitute the characteristic feature of this region and are very numerous. Connective tissue trabeculae are made out with great difficulty in all portions of the gland; the vascularization seems to be that of an irregularly distributed network of fine capillaries, except in the two dorso-lateral projections where the presence of a rich meshing of large and small blood channels constitutes one of the distinguishing features of this region. If the homogeneous clumps observed in this last named region are to be considered as colloid or some allied substance there is an abundance of it present, although nothing of the kind was found in the residual lumen itself. In its development, even as late as the last day of incubation, the infundibular process presents a series of tubules branching out from the main infundibular cavity and carrying with them into the neuroid tissue an open-

dymal lining in much the same characteristic manner as has been observed in some of the lower forms.

Columba livia (fig. 32). The hypophysis of the pigeon presents an infundibular process and a pituitary gland. The processus infundibuli is a relatively large structure, situated in greater part posterior to the gland substance, but also, to some extent, superior to it. Its form is such that it embraces the gland along its dorso-lateral aspects, so that, if viewed from the side, the entire hypophysis would have the appearance of an acorn. A slender stalk connects the infundibular process with the under surface of the inter-brain. The gland lies beneath the processus, is separated from it by a residual lumen, and presents a juxta-neural and distal epithelial portion. The juxta-neural portion is a thin layer investing the infundibular process. The distal portion is of considerable dimensions and posteriorly borders upon a spacious saccus vasculosus. Histologically the pituitary gland consists of two parts, a peripheral and a central area. Although the lines of demarcation are not sharply drawn, the cellular constituency of the peripheral area serves to differentiate it from the deeper lying central portions. The peripheral cells are exclusively of the basophilic type. In their arrangement these cells resemble those of the fowl, being clustered into thick, irregular masses or so collected as to give the appearance of acini. It is possible to discern three distinct areas in the peripheral portion of the gland, namely: the posterior area bordering upon the residual lumen which is characterized by the conspicuous size of the apparent acini, the inferior area which stands out prominently because of the presence in it of many large sized, thin walled blood channels, and a superior portion, a very narrow zone containing a great number of acidophilic cells in addition to the basophiles, showing but few apparent acini. The central portion has much the same arrangement as the other parts just mentioned, except that the acidophilic cells are more numerous. The residual lumen is situated in part above the gland but to some extent posterior to it. The cells bordering upon the lumen in the anterior as well as in the posterior wall are of the basophilic type. The gland is very rich in large blood spaces, especially along its inferior and posterior borders. Connective tissue tra-

beculae were clearly made out in all areas of the body; colloid material was observed in the apparent acini and also to a lesser degree in the residual lumen. Both varieties of acidophiles are present.

Didelphys virginiana (fig. 33). The hypophysis of the opossum presents an infundibular process and pituitary gland; the latter has a distinct juxta-neural and a distal epithelial portion. In position the processus is placed supero-posterior, in greater part resting upon the upper surface of the gland. The juxta-neural epithelial portion of the gland completely invests the infundibular process except for a small region about the proximal part of the stalk. This stalk contains a lumen which is carried downward and backward to the expanded distal extremity of the process. The distal epithelial portion follows the general arching of the juxta-neural portion but is separated from the latter by a wide cleft, the residual lumen. In this manner the juxta-neural and distal epithelial portions as well as the residual lumen encircle the infundibular process. The juxta-neural portion is composed of faintly staining basophile cells densely crowded together in a layer several cells thick. The distal epithelial portion presents two regions in which two varieties of cells are observed. The area bordering upon the residual lumen anteriorly comprises basophilic cells, the faintly staining type being more numerous and arranged in cell masses; occasional clusters of deeply staining basophiles also appear. The remaining area of the distal portion, a wide zone extending out to the periphery, is made up of faintly and deeply staining acidophiles, the former predominating. The arrangement is that of cell cords, masses and apparent acini. Some of the acini have lumina in which a colloid material was observed. Numerous large, irregular blood spaces ramify in the distal portion; connective tissue elements are with difficulty traced into the gland.

Mus decumanus (figs. 34 and 35). In the rat the general relations of the pituitary gland to the infundibular process are well marked. This is another instance in which it is improper to speak of the anterior and posterior lobes of the body with reference to the two portions so denominated in the human anatomy. The processus infundibuli in the rat, and in Rodentia in general, lies as

a small heart shaped lobule upon the superior surface of the pituitary gland, filling a shallow fossa. To the unaided eye, the line of demarcation between the two portions of the body is quite evident, a narrow furrow being interposed between the outer limits of the infundibular process and the inner border of the gland. The derivative of the neural ectoderm is, in reality, a superior lobe, while the glandular portion is an inferior lobe. In the furrow between the two lobes lies the residual lumen whose roof is formed by a thin prolongation of the epithelial structure of the glandular tissue. The juxta-neural epithelial portion surrounds the infundibular process as a deep layer of faintly staining basophiles whose general arrangement is that of an irregular stratum with occasional acini of deeper staining elements. The distal epithelial portion contains both basophiles and acidophiles, the former grouped about the residual lumen and entering into a narrow zone marking the periphery of the gland, the latter occupying the central regions. As already mentioned the residual lumen is present. Its posterior wall is a thick, irregular layer of large basophilic elements. The posterior wall differs somewhat from the rest of the gland because of the scarcity of blood elements; it stains deeply with basic stains. The actual glandular portion is richly interspersed with irregular blood channels containing blood cells. Delicate strands of connective tissue may be traced along the cell cords and around the acini but no colloid material or allied substance was observed in any portion of the gland.

Felis domestica (figs. 36 and 37). The hypophysis in the cat presents a pituitary gland and an infundibular process but no saccus vasculosus. The infundibular process is situated behind and somewhat above the gland whose posterior wall it indents in such a manner as to become embedded in the glandular tissue. The juxta-neural epithelial portion surrounds the infundibular process. In greater part it appears in the form of an attenuated membrane on the border of the processus, made up of basophilic cells. The distal epithelial portion is largely made up of acidophiles arranged in cell cords and apparent acini. Both the faintly and deeply staining cells enter into this arrangement. A narrow peripheral zone of faintly staining basophiles surrounds the large central

area of the acidophiles. Upon reaching the summit of the gland, this peripheral zone becomes much thickened and it is here that the basophiles are found in greatest numbers. A thin layer of faintly staining basophilic cells intervenes between the residual lumen and the central area of the acidophiles. From this layer, near its center, there projects into the surrounding acidophilic cells, a narrow process of basophiles. In this region some intermingling of the two types occurs. The residual lumen is well marked. It presents a typical anterior wall composed of basophilic elements; its posterior wall is somewhat exceptional to the general arrangement observed in this area. The acidophilic areas present a relatively rich vascularity, being supplied by numerous irregular blood spaces. The anterior wall of the lumen shows no increase in this supply over the other adjacent areas, while the actual lining of the posterior wall is absolutely destitute of all signs of vascularity, although numerous large sized vessels are seen coursing in the adjacent areas of the infundibular process. Connective tissue trabeculae were easily distinguished throughout the glandular portions, giving the structures the appearance of irregular lobulation. No colloid or other material could be found in the lumina of the acini or in the ventricle.

Canis familiaris (figs. 38 and 39). The hypophysis of the dog presents an infundibular process and a pituitary gland but no sacculus vasculosus. The infundibular process is situated superoposteriorly and is entirely invested by the juxta-neural epithelial portion except for a small section of its stalk. To about the same extent, the residual lumen also surrounds the processus. In the distal portion the following topographical and cellular areas are differentiated; first a central zone composed chiefly of faintly and deeply staining acidophiles interspersed among which are a few clusters of deeply staining basophiles. The acidophiles are arranged in irregular groups, cords and acini. Second, an apical zone situated at the anterior tip of the gland and made up exclusively of basophiles so arranged that the deeply staining elements in the form of acini are placed upon a background of faintly staining cells. Third, a peripheral zone extending from the apical zone backward along the central area and composed of both acido-

philes and basophiles of the faintly staining type. Fourth, a circumventricular zone, forming the anterior wall of the residual lumen, exclusively basophilic. Here both varieties are found, the faintly staining cells affording a background against which the deeply staining elements, arranged as acini, stand out conspicuously. This latter region is the dorsal continuation of the peripheral zone. In its lateral portions it is thick. In its anterior segments it interposes itself between the central zone and the residual lumen, being a thin layer of flattened basophilic cells. The acidophiles of both types predominate in the central zone. In size they are large elements with clear boundaries and nuclei staining deeply with haematoxylin. They are arranged as cords or cell masses, but in a few instances they are observed to participate in the formation of the apparent acini. The basophiles are also conspicuous elements. They appear as two distinct varieties, the deeply staining cells and the faintly staining cells. The latter cells tend to arrange themselves in cords and masses, while the former type is invariably seen to enter into the aciniform arrangement. The deeply staining basophiles are the largest elements observed in the gland. One of the most interesting features of the body is its residual lumen, especially as to its relations and its limiting walls. The processus infundibuli appears to have made a much more effective impression upon the posterior wall of the pituitary anlage than in the case of any other of the animals observed. In fact, the so-called posterior lobe of the hypophysis indents the posterior surface of the pituitary gland so deeply that processus is practically embedded in the glandular tissue, with the exception of its stalk, which remains free. The residual lumen resulting from these conditions is unusually extensive. Its walls are formed of epithelial cells as in all other cases, but its vascular arrangement differs considerably at different points. The anterior wall presents the most vascular area of the entire gland, a fact which led Löthringer to the belief that the residual lumen serves as a receptacle of the glandular secretion, whence it was absorbed by this rich capillary network, so characteristic of the anterior wall of the ventricle. It is to be remembered, that this fact does not hold good in all species and therefore the idea of Löthringer cannot be

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accepted without qualification. The vascularity of the rest of the gland is relatively rich, except the posterior wall, in which only a few blood-vascular elements are to be found. Connective tissue processes establish a well marked trabeculation and colloid material is present in abundance both in the ventricle and in the acini of the central zone.

Ovis aries (figs. 40 and 41). Peremeschko was among the first to call attention to the chief factor which gives such a striking appearance to the hypophysis of the sheep, namely, the presence of a rich trabecular system dividing and subdividing the structure until it has acquired the semblance of a lobulated organ. The sheep's hypophysis has a processus infundibuli and a pituitary gland but no saccus vasculosus. The juxta-neural epithelial portion surrounds the infundibular process. It presents the most striking characteristics as the posterior wall of the residual lumen of all the tissues examined. Over the ventral surface of the processus, the juxta-neural portion is very thick but becomes greatly attenuated as it passes out over the lateral aspects. It is made up entirely of basophiles. The distal epithelial portion presents a thin circumventricular layer of basophiles forming the anterior wall of the lumen, in front of which is the main body of the gland. The gland is chiefly composed of acidophilic cells, in fact, they are so much in the majority that the other cellular elements appear numerically insignificant. The acidophiles are found in the central zone, and in the anterior portion of the peripheral zone. They are arranged, in part, as cell cords, but their most frequent occurrence is in the typical aciniform arrangement. A very noticeable feature is the entire absence of the cell masses observed in all the other pituitary material examined. In this animal each epithelial cell appears to have its definite place in a distinct cell cord or in an apparent acinus. The general tendency toward syncytial cell massing is entirely wanting. The faintly and deeply staining cells intermingle. All of the acini vary in size; some few present lumina, although the great majority are solid. The basophilic cells are not so numerous, but are conspicuous elements. They appear as the exclusive cells in the posterior peripheral zone, and also in the posterior and lateral walls of the residual lumen. They

are also in a less degree scattered throughout the central zone. The residual lumen in the sheep is very similar to that in the dog, the infundibular process apparently having been invaginated by the posterior pituitary wall. It is with this wall that fusion has taken place. The wall itself is made up exclusively of basophilic cells which arrange themselves as long convoluted cell cords, in many instances possessed of distinct lumina. The lateral walls are likewise entirely composed of basophiles arranged in the same manner. In vascularity the tissue shows a very even distribution of large and small blood spaces; many small vessels appear to enter the gland in company with the connective tissue trabeculae and follow these processes into their more minute ramifications. The trabeculae themselves are more prominent in this animal than in any other; they establish a rich meshing about the apparent acini until the collections of these latter take on the appearance of actual lobulation. The walls of the ventricle offer nothing particular in the way of vascularity and in this respect are different from those of the dog. Colloid or some allied material was observed in the residual lumen.

Cynocephalus babuin (figs. 42 and 43). The hypophysis in *Cynocephalus babuin* is typical in its general make-up, but presents a rather more exact distribution of its cells than is the case in most mammals. The infundibular process is large and attached to a short stalk; in position it is posterior to the gland. The juxta-neural epithelial portion is of irregular thickness, having its greatest diameters in the regions about the residual lumen. It clearly defines itself by the staining properties of its cells, as well as by their arrangement, which in cross section gives the impression of irregular stratification, entirely devoid of apparent acini, cell cords or clusters. This layer forms a partial and attenuated investment about the processus infundibuli. The distal epithelial portion has all the characteristics of this part in the mammal, including the four varieties of cells and their several modes of arrangement. Deeply staining basophiles occur as large elements, having an aciniform arrangement against the general background of the faintly staining basophiles. They occupy the largest portion of an area situated at the center of the gland; extending antero-pos-

teriorly, this area touches the periphery in front and the juxta-neural portion behind; for this reason it may be termed the area centralis. On reaching the anterior periphery of the gland the basophilic cells spread out as a thin circumferential layer which passes insensibly into the juxta-neural portion investing the infundibular process. In this thin layer, which may be called the area lateralis externa, the cells are exclusively of the faintly staining basophilic type; they are arranged in masses, with here and there an occasional aciniform appearance. The acidophilic cells are also of two varieties, namely, those that react intensely to acid stains, and those which, although they give the impression of taking the stains more deeply, actually do so only in their nuclei which are large and reticular; their cytoplasm is scant and faintly acidophilic. These latter elements, the faintly staining acidophiles, constitute about four-fifths of the entire gland but are segregated into two distinct areas, one on either side of the area centralis and bounded externally by the area lateralis externa. In these regions it is much more difficult to distinguish the cell outlines, the cells themselves being small and closely crowded together. They are arranged in dense, convoluted cords but present no apparent acini. The deeply staining acidophiles are much fewer in number but, because of their brilliant staining, stand out vividly amongst the more numerous cells of the other acidophilic type. They occur as two or three narrow zones, completely surrounded by the faintly staining acidophiles from which latter they differ in size, being much larger elements, with more definite limits and very small picnotic nuclei. Their arrangement is also dissimilar, as they appear to be solid cell masses, without any tendency toward cord formation or convolutions. The juxta-neural epithelial portion is an irregular zone between the processus infundibuli and the distal epithelial portion. In some places it is of considerable thickness, in others reduced to a narrow stratum. It surrounds the residual lumen in the regions where this cavity appears and then extends backward as a thin layer to form a partial investment about the infundibular process. The cells of this portion are exclusively faintly staining basophiles. They are smaller than the basophiles found in other areas of the gland, having smaller nuclei and poorly

defined cell boundaries. The residual lumen is asymmetrically placed and of irregular outlines. Its walls, although of some thickness, show no aciniform arrangement of cells and no blood spaces or vessels. The gland of this animal shows a large amount of connective tissue which, in the form of slender, branching trabeculae, tends to subdivide the structure into lobules. This feature is most marked among the acidophilic cells where the blood spaces are also largest and most numerous. Smaller blood spaces were observed in the basophilic areas but no colloid material was found in any portion of the gland. The striking feature in the tissue of the organ is the well defined separation of the several types of cells into districts of their own.

Human embryo of 20 mm. (fig. 44). Once established within the cranium and completely separated from the mouth cavity, the saccular anlage of the hypophysis in the human embryo begins to take on the following characteristic changes:

1. Adaptation of the sac to the sella turcica with the consequent formation of two long walls, one ventral, the other dorsal, and two narrow side walls.

2. The dorsal wall along the median line is in relation with the now attenuated prolongation of the diencephalon, the infundibular process, and thus becomes the juxta-neural epithelial portion.

3. The anterior wall or distal epithelial portion, in the regions nearest the mouth, presents a large median evagination and two smaller lateral ones. In these evaginations and regions of the anterior wall intervening between them, there is evidence of rapid cell multiplication, taking form in irregular cell masses or definitely aciniform clusters projecting ventrad from the residual lumen. The posterior wall, very little if any thickened, shows no such tendency to rapid cell increase. At the point where the anterior and posterior walls meet the cells are also seen to be actively proliferating.

4. The lumen has been reduced to a transverse slit, due to the antero-posterior flattening of the original sac.

It will be seen that the posterior wall, having acquired its juxta-neural relations, remains practically quiescent, while the anterior or distal epithelial portion, in great part, contributes to the growth

of the gland. All of the cells in either wall are deeply staining basophiles. They are arranged as irregular masses and as cell cords in the anterior wall, and a similar arrangement, although on a much smaller scale, is seen in the posterior wall. No blood spaces or vessels can be detected, nor were any connective tissue elements or colloid material observed. There is no evidence yet of the posterior wall assuming the typical relations of more advanced stages, although it is not difficult to appreciate the process by which the juxta-neural portion will come to form an investing layer of epithelial cells about the infundibular process.

Human fetus of ten weeks (fig. 45). The advance from the late embryonic stage as seen in the embryo of 20 mm. consists largely in the more exuberant growth of the anterior wall of the residual lumen, i.e., the distal epithelial portion. This growth has now been carried to such a point that the body assumes an oval outline with its long axis extending antero-posteriorly. As compared with the juxta-neural portion, the volumetric increase has been about six to one; some proliferation has occurred in this portion as well; this is witnessed by the invasion of the neuroid tissue of the processus by irregular cell masses from the posterior wall of the lumen. The cells in all parts are exclusively of the basophilic type, both varieties, the faintly and deeply staining cells, being present. In arrangement the deeply staining cells stand out as convoluted cords against a background of more faintly staining elements. The residual lumen is still a spacious cavity. No blood spaces, connective tissue, or colloid material were observed.

Human fetus of twelve weeks (fig. 46). It must be evident from the facts already given that the essential change in the hypophyseal structure in ascending from the lower to the higher forms is the metamorphosis of the cell cords or chains into a more complex arrangement depending upon the simple convoluting of these cords. These latter appear to have a tendency to acquire lumina. In the fetus of the twelfth week the cord stage is still fairly well marked; at the same time there is present a noticeable disposition toward convolution. The gland itself at this stage shows no evidence of any particular morphological or topographical divisions, such, for instance, as were observed in several of the lower species exam-

ined. Its cells are wholly of the basophilic type. They are large and well defined. The juxta-neural portion, a stratum several cells thick, invests the infundibular process; the distal epithelial portion lies ventral to the residual lumen. The basophiles are of the faintly and deeply staining varieties, the former being in the majority. The narrow cleft marking the residual lumen in the posterior regions begins to expand rapidly as it extends to the front and finally becomes a wide sinus with undulating walls. The cells of the anterior wall appear to take the basic stains most intensely, especially those cells bordering immediately upon the ventricular space. It is to be remembered that the cells in the anterior wall alone are active in the formation of the cell cords which later on, by a process of convolution, give rise to the apparent acini. The posterior wall retains its early shape and has but few acini.

Human fetus of fourteen weeks (fig. 47). The results of the metamorphosis from the cell-cord stage to that of the apparent acini are first observed in the human fetus at the fourteenth week. Here the great majority of the glandular cells have arranged themselves in aciniform groups or are displaying a distinct tendency in that direction. A smaller number are grouped in irregular cell masses which furnish a background for the aciniform collections. The entire absence of all cellular elements except the basophiles is still characteristic; two types of these may be distinguished. The cells in the aciniform arrangement, or showing a tendency in this direction, stain very deeply both in the cytoplasm and in the nucleus, while the elements which tend to form the cell masses stain faintly. The greater number of the acini present large lumina, although some remain solid. Some wide blood spaces are found in the glandular areas, especially in the proximity of the apparent acini. Their walls, however, are difficult to make out and very few blood cells were observed in them. The residual lumen in its general relations and arrangement remains unchanged. No connective tissue trabeculae and no colloid or allied material could be found in any part of the gland.

Human fetus of twenty weeks (fig. 48) The histological findings in the human fetus of the twentieth week summarize the conditions

of the earlier stages so clearly as to call for a detailed account. The material of this age was examined in sagittal planes, serial sections, including the entire head. In the general relation of its parts, the hypophysis at this stage approximates adult conditions. The processus infundibuli has attained greater dimensions and lies almost entirely posterior to the glandular part. The pituitary gland has likewise increased in size; its longest diameter is now antero-posterior, parallel with the floor of the skull. The juxta-neural epithelial portion almost completely invests the infundibular process, sending a thickened projection upward along the front of its stalk. Its cells are exclusively basophilic. The distal epithelial portion has increased greatly in size and is continued forward in a tongue-like process toward the optic chiasma. Histologically the gland is composed exclusively of a single type of cells, the basophiles. Of these latter two varieties are observed, the darkly staining and faintly staining cells. No acidophiles can be found in the gland. The cellular arrangement bears out the early stages. Cell cords, cell masses and apparent acini appear in the several parts of the gland tissue, the predominating arrangement being the apparent acini. These acini are made up of the basophilic cells which stand out as thickly clustered clumps against a background of the faintly staining basophiles arranged in cell masses. Along the periphery of the gland the apparent acini are much fewer in number, the cells arranging themselves in thick, irregular cords which skirt the borders of the body. The cellular arrangement in the anterior wall of the residual lumen is somewhat different from the other portions already described; here the apparent acini cease to be the predominating feature; the cells appear in dense, irregular masses, basophiles of both varieties being intermixed in about equal proportions, with here and there an apparent acinus. The cells forming the actual lining of the lumen are exclusively of the deeply staining type, being cuboidal in form and arranged in a shallow stratum (two or three cells deep). If anything, the internal limiting stratum of the posterior wall is somewhat thicker than that of the anterior wall and its general outlines are much more irregular. This posterior wall presents not a few evaginations of varying sizes which extend backward into a thin layer of faintly

staining basophiles. In some instances, it appears that the cells from these evaginations have left their original positions and are invading the territory of the processus infundibuli. Such cells arrange themselves in irregular cell masses and are always of the faintly staining type. From a study of this stage it is possible to give a definite description of the mode of origin of the connective tissue trabeculae which form such a dense network throughout the gland. In the mesial planes a thick, short stalk of connective tissue is observed to descend from the under surface of the diaphragma sellae and then almost immediately to penetrate into the pituitary tissue as far as its center. At this point the stalk rapidly breaks up into numerous thin strands which stream out in all directions and may be followed out to the periphery. Near the center of the stalk small vessels are to be observed, which may be traced into the gland to the point where the stalk begins to break up. Further than this the blood channels cannot be followed very clearly. In part they seem to accompany the radiating trabeculae, but only for a short distance, very soon turning inward to ramify among the cells, entirely independent of the connective tissue. These vascular channels seem to open out into broad blood spaces or lacunae, whose walls are with difficulty recognized; their actual boundaries seem to be the cells of the gland. At this stage the blood spaces have already made their appearance in considerable numbers. They are still relatively few when compared with the vascularity observed in the eighth month fetus, the eighth month infant and the adult gland. No colloid material was found in any part of the glandular tissue.

Human fetus of six and seven months (figs. 49 and 50). In both of these stages the conditions as to relative proportions, arrangement and cellular constituency have undergone but little change. The juxta-neural epithelial portion has made a somewhat higher ascent along the stalk of the infundibular process in the seven month fetus, while the posterior wall of the residual lumen is also a little thicker. The gland is still exclusively composed of basophiles.

Human fetus of eight months (fig. 51). The epithelial unit of structure is still the basophilic cell; the structural design, with the

exception of a few minor details, is essentially the same as that of the ten week fetus. The processus infundibuli has attained considerable dimensions and now lies entirely behind the glandular portion. It still bears a ratio of about one to six. Between the two lobes of the body the characteristic residual lumen or ventricle interposes itself. The rapid increase of vascularity throughout the glandular portion does much to give the body a considerably different aspect from the picture presented in the earlier stages. At this stage the gland has become a very vascular organ, more so perhaps, than at any previous or subsequent stage of development; much of its bulk is due to the dense network of large and small sized vessels within whose meshes the epithelial elements are lodged. There seems to be little disposition for this vascular supply to affect one area or portion of the gland more than another, except for one particular region which will be spoken of later. The residual lumen seen in transverse section at this stage presents some noteworthy features; chief among these is the arrangement of the basophilic cells as long cell cords with distinct lumina. The epithelial cells of the juxta-neural portion are arranged in three layers which may be easily differentiated because of the different degrees of intensity with which they take the stain. The layer bordering immediately upon the ventricular space consists of a single row of low columnar elements whose protoplasm and nuclei stain deeply; the long axes are at right angles to the residual lumen. The next stratum comprises cells, larger both in their nuclei and cytoplasm, but staining less intensely. This layer is several cells deep and in it are lodged the cell cords already mentioned. These cords stand out prominently because their cells are of the deeply staining type. The third stratum comes into direct contact with the processus infundibuli; its cells resemble those of the first layer in character and arrangement, although they form a border line that is not regular; numerous epithelial projections of various sizes penetrate into the neuroid tissue of the infundibular process. The vascularity of this posterior wall of the ventricle is relatively richer than that of any other area, the vessels appearing to run parallel to the residual lumen. Every section shows the presence of colloid material in the ventricle.

Human fetus of the ninth month (fig. 52). One essential element of advance occurs in this stage, namely, the introduction of a small patch of acidophilic cells deep in the central region of the gland immediately in front of the residual lumen. The genesis of these cells is not clear. In them and the adjacent basophiles there are many figures suggesting mitosis, yet the cells themselves have such scant cytoplasm and are so densely crowded together, that it is impossible to state with assurance that the figures are actually those of division. Another change is the marked increase in vascularity in this acidophilic area. It has been a feature of the gland, in the early stages, to present an evenly distributed vascularization. But the disparity becomes so marked in this stage as to make it the characteristic of this new acidophilic area. The blood spaces are larger as well as more numerous. This stage must represent the transition from the monocellular type to that of the two-cell variety, i.e., with both acidophiles and basophiles present, the latter still greatly in the majority.

Infant of eight months (figs. 53 and 54). The decisive change noted in the fetus of nine months is further emphasized in this stage by the great increase in number of the acidophilic cells. The composition of the gland at this time is of such complexity as to require description. The infundibular process is present, but has, to a great extent, outgrown its former dimensional disparity; it is now a little less than two to one with reference to the glandular portion. The pituitary gland proper is situated anterior to the processus infundibuli and appears in the form of a single, solid lobe with well marked juxta-neural and distal epithelial portions.

In tracing the distribution of the several cell types it is possible to recognize four distinct areas in which the cells tend to group themselves more or less discretely. The first region is that of the juxta-neural epithelial portion, composed exclusively of faintly staining basophilic cells, arranged in an irregular stratum and of slight vascularity. The second area lies in front of the residual lumen, forming a central zone which reaches the periphery anteriorly. It is composed of faintly and deeply staining basophiles with here and there a small cluster of acidophiles. This region is very vascular and contains the largest blood spaces found in the

gland. The third region consists of two lateral projections, lying one on either side of the central zone and composed of deeply and faintly staining acidophiles. An occasional clump of basophiles was also observed. The fourth area constitutes the narrow peripheral zone which, starting at the anterior margin of the central zone, passes along the outer edge of the gland to become continuous with the juxta-neural portion. The cells here are for the most part of the faintly staining basophilic type, with some deeply staining acidophiles interspersed. In their general characteristics the basophilic cells are similar to the corresponding elements observed in other species and in the younger stages of man. They appear as two well differentiated types. Those which stain more intensely have a decided tendency to arrange themselves in the form of apparent acini; the cells of the second type are arranged in large, irregular masses. The acidophiles have a much more ample cytoplasm than the basophiles and their limits are more clearly marked. Their general arrangement is similar to that of the faintly staining basophilic elements; they string themselves out in long, irregular and much branching cords, each cord being of considerable thickness. Among them an occasional basophilic acinus is found. The two lateral acidophilic areas terminate abruptly at their internal limit which marks the transition into the basophilic, central zone; at their external boundary, the basophilic peripheral zone begins.

Not only are the two distinct cell types present at this stage, but each cell type has its particular areas of distribution. The residual lumen still persists as a wide cleft; the general arrangement of its walls remains essentially the same. The anterior wall, except in the central regions, is composed of several layers of faintly staining basophilic cells. At the center, however, the wall becomes suddenly thickened, forming a more or less circular area which dips into the glandular tissue as if to surround the ducts or tubules that seem, at this point, to be centering in toward the ventricle. The posterior wall, forming the juxta-neural portion, has undergone some changes since the stage of the nine month fetus; chief among these is the disappearance of its intermediate layer and its replacement by a narrow band of connective tissue. In the cen-

tral regions the epithelial cells are crowded in a dense, irregular stratum with an occasional cell cord. In this area the connective tissue is much denser; the blood vessels are larger and more numerous than elsewhere in the gland. Connective tissue trabeculae are easily made out in all parts of the gland, intersecting each other in such a way as to divide the cellular elements into large and small collections, and giving the tissue the appearance of a lobulated organ. Large collections of colloid material were found in the residual lumen.

Human adult (figs. 55, 56, 57, 58, 59 and 60). The adult hypophysis in man becomes much easier to interpret in the light of the lower forms and fetal stages already discussed. Guided by this knowledge, it is possible to establish a reliable basis of analysis by which the morphological elements of the gland may be tested. The hypophysis in man presents two characteristic lobes or divisions with reference to their derivation, i.e., the processus infundibuli, derived from the neural ectoderm, and the pituitary gland, derived from the oral ectoderm. Fused in such a manner as to form a single body, the hypophysis cerebri is situated in the deep recess of the sella turcica. The two portions just mentioned are most frequently spoken of by anatomists as the anterior and posterior lobes, a description which in man accurately defines the relative positions of these two divisions. In the earlier stages the infundibular process was seen to produce a fossa in the posterior wall of the glandular anlage, which concavity persists for some considerable time after birth but disappears when adult life is reached. With these minor exceptions the relations of the two parts of the body are the same as already observed. The juxta-neural and distal epithelial portions are well defined in the usual manner. The cellular differentiation is also evident and especially well marked in the distal portion. The basis for the differentiation arises from the manner in which the two types of cells tend to separate themselves into distinct areas of their own. A large, triangular area, situated dorso-superiorly, is made up almost exclusively of the acidophilic cells, while an even larger area, situated below and to the front, comprises quite as exclusively the basophilic elements. In addition to this discrete distribution, a narrow band of baso-

philic cells encircles the entire glandular portion, becoming somewhat attenuated as it passes beneath the acidophilic area. Two varieties of basophiles may be distinguished: (1) the large sized, deeply staining cells, and (2) the medium sized, faintly staining cells. The large sized basophiles seem to have their prototypes in the deeply staining elements so frequently referred to in the description of the lower forms. Their protoplasm is extensive and homogeneous, their nuclei are relatively small and their boundaries clear. In general outline they are polygonal, but when seen in their typical formation they appear to be columnar. These cells are found exclusively in the distal epithelial portion; they arrange themselves as dense cords and very often have all the appearance of the apparent acini. Their number is relatively large and they present themselves as the most prominent feature of the upper epithelial portion, standing out against the background of the less deeply staining cells. The medium sized cells are easily distinguished because their nuclei as a rule are large and granular, having a distinct nucleolus; their protoplasm, on the contrary, is proportionately small and takes the stain feebly. They have no definite arrangement but serve as ground work for the large sized cells; they occur exclusively in the juxta-neural epithelial area, in the central and peripheral zones. Of the acidophilic cells which form the chief constituents of the distal epithelial portion, two types were observed: (1) the large, deeply staining acidophiles, and (2) the small faintly staining acidophiles. The former elements are relatively few in number but, whenever seen, appear as large cells whose protoplasm stains deeply. The limits of these cells are well defined; they are distributed irregularly, intermingling with the small elements of this area to form dense and long cell cords. The smaller acidophiles have a small amount of cytoplasm. Their nuclei take the haematoxylin more uniformly than in the case of the larger cells; they also present a nucleolus. A singular feature about the distribution of both varieties of these cells is the manner in which they are prevented from coming in actual contact with the infundibular process, for although the superior epithelial portion is in close proximity to the posterior lobe, a band of basophiles is interposed between them and the latter

structure. Another feature is the disposition of the residual lumen with reference to the acidophilic area of the gland. The ventricle is well marked between the juxta-neural epithelial portion of the gland and the distal portion along the under surface; it becomes obliterated as the level of the stalk is approached. The ventricle, therefore, has become somewhat modified since the earlier developmental stages; the apparent cause of this modification seems to be the vast increase in the number of acidophilic cells. As already stated, the residual lumen is no longer interposed between the entire glandular portion and the posterior lobe, but appears only as a space between the inferior areas of the distal portion and the lower region of the infundibular process. That part of the ventricle, which does remain, is typical in all respects. It presents a wide lumen whose anterior wall is formed by a thin stratum of basophilic cells. On approaching the lower limits of the hypophysis this wall is thrown into numerous deep folds, as if to enlarge the chamber of the ventricle from below. These folds also extend backward along the under surface of the infundibular process for some distance. The posterior wall of the lumen is composed of a thick layer of medium sized, faintly staining basophiles many layers deep, which extend for greater or less distances into the neuroid tissue of the posterior lobe. A thick strand of these cells can be traced along the under surface of the processus infundibuli, even as far back as its dorsal extremity, at which point several well defined collections of the large sized basophiles have been observed. A similar, but more narrow strand, may sometimes make its way into and directly through the posterior lobe. In its vascularity the anterior wall of the residual lumen is especially rich, although the posterior wall presents some blood channels of larger calibre. The vascular elements in the substance of the gland seem to be more in the character of blood spaces than true vessels; they have irregular boundaries both in transverse and sagittal section. The greatest vascularity is found in those regions in which the acidophiles predominate; the vessels here are not only more numerous but also much larger. In the peripheral zone the vascular elements are somewhat less prominent than in the other basophilic areas. The presence of colloid material is characteristic

throughout the basophilic areas, especially in the apparent acini of the peripheral and central zones; it is also observed in even larger quantities in the ventricle, and appears in the numerous side pockets of the residual lumen. As to the connective tissue, the human pituitary shows much trabeculation. This is particularly the case in the peripheral zone where the processes of connective tissue mark off the acini with great distinctness.

A complete summary of the histological observations will be found in the appended, comparative tabulation.

REPORTS OF SIX HYPOPHYSECTOMIES PERFORMED UPON CATS

The writer has selected the six cases which follow from a series of eighteen hypophysectomies performed upon cats and dogs. The majority of the animals died shortly after the operation. Such cases have been excluded from this report because death was, in every instance, due to some accident of the operative procedure. Only those cases which survived the operation for some considerable time will be cited.

Case 1. Cat, female; 2560 grams, lived nine days. Cause of death, septic pneumonia. Hypophysis completely enucleated.

Case 2. Cat, male; 3000 grams; lived fourteen days; great emaciation; no other symptoms. Cause of death, basal meningitis. Hypophysis completely enucleated.

Case 3. Cat, male; 3200 grams; lived twelve days. Sudden death in dyspnoea. Cause of death, purulent meningitis. Hypophysis completely enucleated.

Case 4. Cat, female; 2800 grams; lived fifteen days. Aggravated cerebellar symptoms. Death in opisthotonos, in tonoclonic spasms. Cause of death, purulent infection of both internal ears and along base of brain. Hypophysis completely enucleated.

Case 5. Cat, male; 3000 grams; lived fourteen days. Great disturbance of gait. Death in prolonged dyspnoea. Cause of death, injury and subsequent infection of pons. Hypophysis completely enucleated.

Case 6. Cat, male; 4150 grams; some changes in disposition, otherwise no symptoms. Killed at the end of sixty-five days. Hypophysis completely enucleated.

It will be seen that five of the six hypophysectomies resulted fatally and in each case the cause of death was an infection following operation. It must be admitted that the older operative method

devised by Vassale and Sacchi⁶⁵ was employed in this series. There can be no doubt that the pharyngeal route offers every opportunity for infection and is to be avoided if possible. No one who has compared this method with that of Paulesco (temporal route) can deny that the latter is the safer from the standpoint of aseptic surgery. Much as it has to recommend it, Paulesco's method is not above criticism. Gemelli⁶³ has attempted to control the two methods and his views are interesting (page 21).

One of the animals (Case 6) of the writer's series survived for sixty-five days, showing no post-operative symptoms other than a change in disposition and loss of weight. In the first two weeks it lost 1850 grams, but subsequently regained this and a little more. The animal unfortunately acquired an unmanageable infection over the left flank and for this reason was killed. It is impossible to draw any conclusions as to the specific effects of hypophysectomy from this series of cases. As a commentary on the method of Vassale and Sacchi, however, the series is significant, for it points to many of the complications which may arise in the post-operative period to obscure the real issue and produce a train of symptoms dependent upon causes other than the removal of the hypophysis.

SUMMARY

1. THE PHYLETIC CONSTANCY OF THE HYPOPHYSIS

In this particular there is a striking unanimity of opinion. Rathke stated that the gland is present in all sauropsids and mammals. According to Müller the hypophysis is essentially the same from myxine to man. No craniate has been reported which has not presented an hypophysis. The work of Andriezen is highly suggestive as to the probable phylogenesis of the organ. In the bucco-neural duct and subneural gland of *Amphioxus* and some larval forms of the lower craniates, he sees the homologue of the hypophysis. His contention, that the group of nerve cells at the superior orifice of the duct, corresponds to the neural portion of the organ and fills an office similar to the ganglia of the osphradial organ in mollusca, has all the faults of deduction based on analogy.

Berkley in part agrees with Andriezen. He believes that the neural portion of the body represents an original special sense organ, lying quiescent but not atrophied. The contributions of these investigators are distinct acquisitions. Their value as evidence, however, will be increased when the findings have been corroborated by other workers.

2. ORIGIN OF THE HYPOPHYSIS

The view that the hypophysis springs entirely from the brain seems altogether untenable. This is also true of the opinion which regards the body as derived from the cephalic end of the notochord. Reichert's contention that the hypophysis takes origin in the meninges has found no supporters. It is to Rathke that we are indebted for the first description of the derivation of the organ. There can be little question that the infundibular process takes origin from the brain, while the glandular portion appears to arise from the epithelium of the mouth cavity. Whether this simple description of the two-fold origin is complete enough to include all of the elements entering into the hypophysis is still a question. It is safest to take the position advocated by Caselli, that it is not possible, for the moment, to state that the organ is exclusively of ectodermic origin.

3. HISTOLOGICAL CHARACTER

Two varieties of cells have long been recognized in the gland, the chromophobes and chromophiles. This nomenclature is based upon the relative and not the differential stainability of the cells, and has consequently led to much confusion. The terms are especially unfortunate in that they may not be applied in the comparative histology of the gland, since twenty-five per cent of the animals examined by the writer do not present chromophobes. Yet in this twenty-five per cent there is a decisive cellular differentiation, based upon the differential stainability of the cells with reference to the acid and basic stains.

In all species examined basophiles and acidophiles were observed. These terms are suggested as being more comprehensive and exact.

It is not until the higher sauropsids are reached that the true chromophobes make their appearance. Too great stress has been laid upon the relation of the chromophiles to the chromophobes, the latter having been considered as functional phases of the former, or even as pathological. This last idea finds no substantiation in the hypophysis of the higher mammals, while the first is immaterial when compared with the fact that there are, throughout the series, two definite types of cells, the acidophiles and the basophiles. These two types tend to keep themselves separate.

The juxta-neural epithelial portion is invariably basophilic as is the anterior wall of the residual lumen (when the cavity is present); certain peripheral and central areas of the distal portion are also basophilic. The intimate relation of the acidophiles to the blood spaces, and of the basophiles to the residual lumen and infundibular process suggests a possible difference in the *modus operandi* of the two types of cells. The acidophiles may contribute their products directly to the blood; the basophiles, by way of the residual lumen and the infundibular process (with its branched tubular prolongation from the infundibular cavity in the lower forms), may add their secretions to the cerebro-spinal fluid.

The character and relations of the *processus infundibuli*, in the lower forms are especially suggestive. The process here is honeycombed by tubular prolongations from the infundibular cavity and these tubes, surrounded by cells from the glandular portion, afford a possible communication with the chambers of the brain. This condition appears in the eel and the gar-pike. It is noteworthy also that the cells which are intimately related to the *processus* are the basophiles; the acidophiles, collected in the more distal portion, are in relation with the large blood spaces. In two other instances this tendency toward branching out into the *processus*, manifested by the infundibular cavity, is also witnessed, namely, in the alligator and the chick in last days of incubation. There are also examples in which the cavity of the infundibulum is brought into the closest possible relations with the gland portion. Thus in *Menobanchus*, *Diemyctelus* and *Eutaenia sir-talis* only a thin wall intervenes between the gland cells and the

infundibular chamber. Here, as elsewhere, the surrounding cells are of the basophilic variety.

This close proximity of the gland cells to the third ventricle or their easy accessibility to it through a tubular processus becomes still more suggestive when it is considered that the investing basophilic elements have little or no access to the blood channels. On the other hand, the acidophiles, from the lowest forms upward, have intimate relation with the blood spaces. It is not yet entirely clear by what means the basophilic products in the higher forms reach the ventricular system of the brain. Colloid substance has been observed in the loose reticular regions of the processus infundibuli, as if possibly en route to the cavity of the infundibulum. This question needs more detailed investigation and preparations for this purpose are now being studied by the writer for a further communication.

The ventricle or residual lumen of the hypophysis has been described by many writers. Gemelli believes it is an artifact. Peremeschko claims that it is to be found in all animals including man. Löthringer interprets it as the collecting chamber into which the products of the gland's secretion are emptied and from which these products are absorbed by means of a rich vascular plexus in the walls of the ventricle. Haller believes that the lumen has a direct communication with the subarachnoid space, and that the glands secretions are thus thrown directly into the cerebrospinal fluid. The writer has observed the residual lumen in all species examined except in *Petromyzon*, *Lepidosteus*, *Anguilla*, *Diemycetus*, *Menobranchus*, and *Rana*. In every instance the walls of the lumen were composed of basophilic cells. The anterior wall, in the majority of cases, was of much greater thickness, although in several notable instances the reverse was true (e.g., in the sheep). The posterior wall often gave the appearance of an attenuated membrane, but as a rule it was represented by an epithelial partition several cells deep. No statement can be made to comprehend the general characters of the cellular arrangement in the two walls, for in both the writer has observed cell masses, cell cords and apparent acini. The vascularity of either wall was variable in the several species observed, in some cases being rich

and, quite as often, almost negligible. For this reason it seems difficult to agree with Löthringer's proposition that the colloid secretion of the gland is absorbed from this chamber by a dense and rich capillary network lodged in the posterior wall. No attempt has been made to verify Haller's belief in a communication between the lumen and the subarachnoid space. In view of the fact that it is not an infrequent finding to observe colloid in the residual lumen, the question as to its source naturally arises. It may, of course, be thrown into the ventricle by the cells immediately surrounding that space. Very often in the human hypophysis, the lumen is not only seen to be distended by large masses of colloid, but its walls are evaginated so as to give rise to cysts of varying sizes, all containing colloid. One of the first requisites in deciding this question would be the proof of a system of ducts leading from the gland tissue into the lumen. Finally, the problem of the residual lumen very properly raises the query as to whether or not the colloid material is a product of the activity of the gland. To designate it the chief product simply because it is found in the glandular tissue seems to be an altogether unwarranted. Its peculiar position in the tissue seems to invalidate this view. In the gland substance the colloid material is invariably found at the center of the apparent acini and hence at the points most remote from the blood spaces. From this point of view, it would appear that the colloid was avoiding rather than seeking the blood stream. Thaon reports the finding of colloid in the blood capillaries, but the writer has not observed it except in the areas above mentioned.

4. PHYSIOLOGICAL EXPERIMENTATION

The removal of the thyroid gland in the attempt to prove that it bears a vicarious relation to the hypophysis has netted indecisive results. Observations upon the nitrogen and phosphorus balance of animals under the influence of pituitary feeding have furnished some interesting statistics. Their value to the general problem is, however, not great, inasmuch as they represent isolated studies upon a single substance, without offering any basis for comparative figures. Injections of animal extracts, among them

pituitary extract, are doubtless of much value, although they have contributed little that is determinative. The best statement which advocates of this method may venture is that they obtain certain results from the injection of the extracts of a particular gland which may or may not be similar to the glandular activity itself. All of the effects obtained from pituitary injections show an apparent influence of the latter upon the rate and force of the heart beat with an attendant alteration of the blood pressure. Even in these respects there are considerable differences of opinion among the several writers.

A brief review of the results obtained from hypophysectomy makes it clear at a glance that there are two distinct classes of investigators whose opinions are directly opposed. (See summary page 22). One group contends that the hypophysis is indispensable to life, its removal causes a definite symptom complex and rapidly leads to death. The other group is opposed to this view, some of them going so far as to say that the hypophysis is entirely rudimentary and without functional significance. Taken at their face value, the observations of these two groups of investigators would indicate that some species and even some members of many species may survive after the removal of the hypophysis. Much stress has been laid upon the symptom complex resulting from hypophysectomy, including cachexia, motor disturbances, alternating anorexia and polyphagia with persistent loss of weight, attacks of dyspnoea, polydipsia, polyuria and psychic depression. It seems impossible to say how much of this complex, even if invariably present, must be attributed to the removal of the hypophysis and how much to a serious trauma which opens the ventricular system of the brain and permits of a complete drainage of the cerebro-spinal fluid. To this danger should be added the probability of injury or infection to the tuber cinereum, the pons, the posterior perforated space, the cerebral peduncles and the infundibular region. Until the several functions of the parts just mentioned are much better understood than at present, there should be considerable hesitancy in attributing symptoms to this or that portion of a very small operative field, whose narrow confines lodge so many important structures. The results

of hypophysectomy will acquire a greater significance when repeated operations upon the several parts of this small field have fixed more definitely the responsibility of the organ.

5. CLINICAL OBSERVATIONS

From the standpoint of the clinician there appear to be excellent reasons for connecting certain pathological conditions of the hypophysis with acromegaly. It must not be forgotten, however, that the mere observation of certain changes in the organ does not of itself establish any etiologic relations between these changes and the diseased condition. The alterations in the hypophysis and the external manifestations of acromegaly may be isolated, unconnected abnormalities. It has been shown that out of sixty reported cases of acromegaly, 61 per cent gave no symptoms of hypophyseal tumor or enlargement (Arnold¹⁰⁸), that changes in the hypophysis may occur in the course of some acute and chronic diseases (Thom¹⁰⁹), that the pathology of acromegaly includes many other organs and systems besides the hypophysis and bones (Arnold), that changes in the bones and soft parts have been observed as secondary to certain respiratory and circulatory affections (osteo-arthropathie hypertrophiante pneumique of Marie¹¹⁰), and that, as is a very general neurological observation, tumors of the hypophysis exist without any symptoms of acromegaly. For these reasons there should be much reservation in attributing acromegaly directly to pathological changes in the hypophysis.

It is evident that great difference of opinion has been expressed in all the more important questions of the pituitary problem. Morphology, however, has proved the phyletic and histological constancy of the organ in craniates. These two established facts seem to warrant the conclusion that the hypophysis is functionally active. The significance of the neural lobe (infundibular process) has not yet been determined. On the other hand, comparative histology makes it appear that the pituitary gland has a two-fold activity, one part dependent upon the basophiles, the other upon the acidophiles. It also offers some evidence that the secretory product of the basophiles passes into the cerebro-spinal fluid, while that of the acidophiles enters the blood.

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PLATES

ANNOTATION OF LEADERS IN ALL FIGURES

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|------------------------------------|--|
| 1 Mouth pouch. | 13 Branched tubular processus infundibuli. |
| 2 Processus infundibuli. | 14 Saccus vasculosus. |
| 3 Mouth. | 15 Deeply staining acidophiles. |
| 4 Diencephalon. | 16 Faintly staining acidophiles. |
| 5 Juxta-neural epithelial portion. | 17 Deeply staining basophiles. |
| 6 Distal epithelial portion. | 18 Faintly staining basophiles. |
| 7 Residual lumen. | 19 Sella turcica. |
| 8 Acini. | 20 Colloid material. |
| 9 Cell cords. | 21 Invading basophiles. |
| 10 Blood spaces. | 22 Tongue. |
| 11 Area lateralis. | 23 Pituitary stalk. |
| 12 Intercardal blood spaces. | 24 Roof of mouth. |

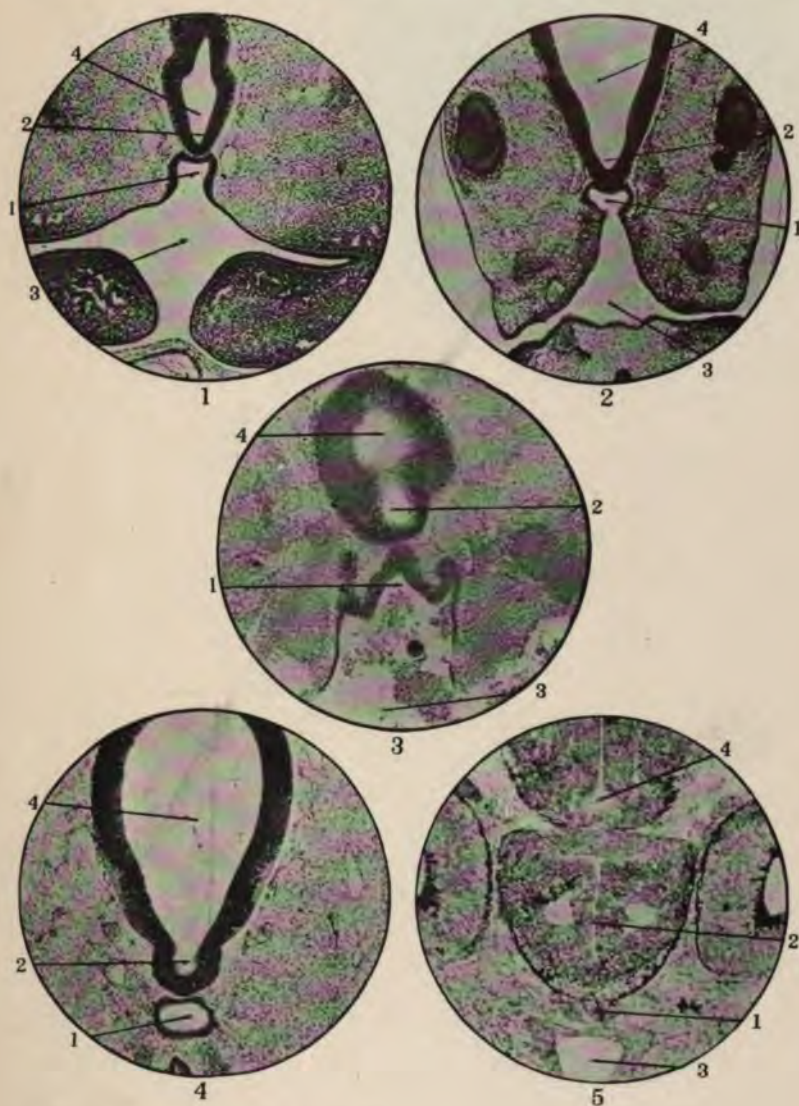


Fig. 1. Cat embryo 6 mm. $\times 50$.
Fig. 2. Chick embryo 8 mm. $\times 50$.
Fig. 3. Aspidonectes embryo 8 mm. $\times 65$.
Fig. 4. Chick embryo 8 mm. $\times 50$.
Fig. 5. *Rana sylvatica* embryo $\times 115$.

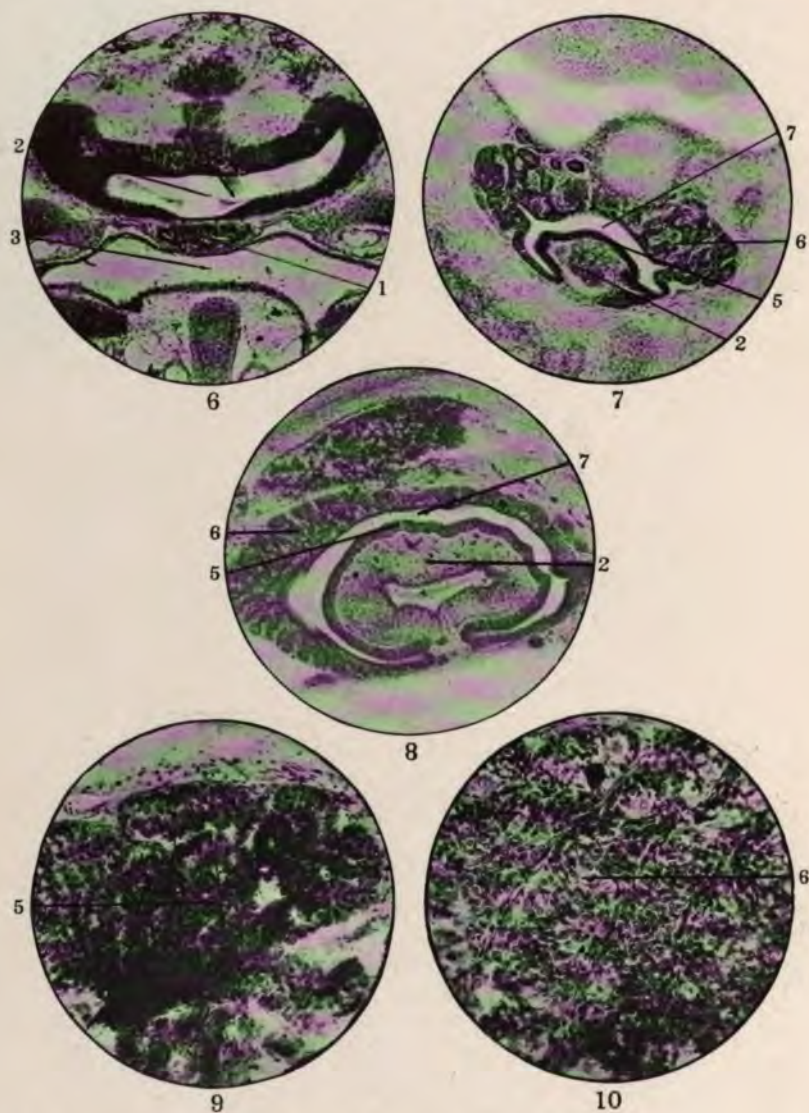


Fig. 6. *Amia Calva* embryo 7 mm. $\times 115$.

Fig. 7. *Tupaia* embryo 15.5 mm. $\times 115$.

Fig. 8. Cat fetus 70 mm. $\times 70$.

Fig. 9. *Petromyzon fluviatilis*. $\times 150$.

Fig. 10. *Petromyzon fluviatilis*, $\times 115$.

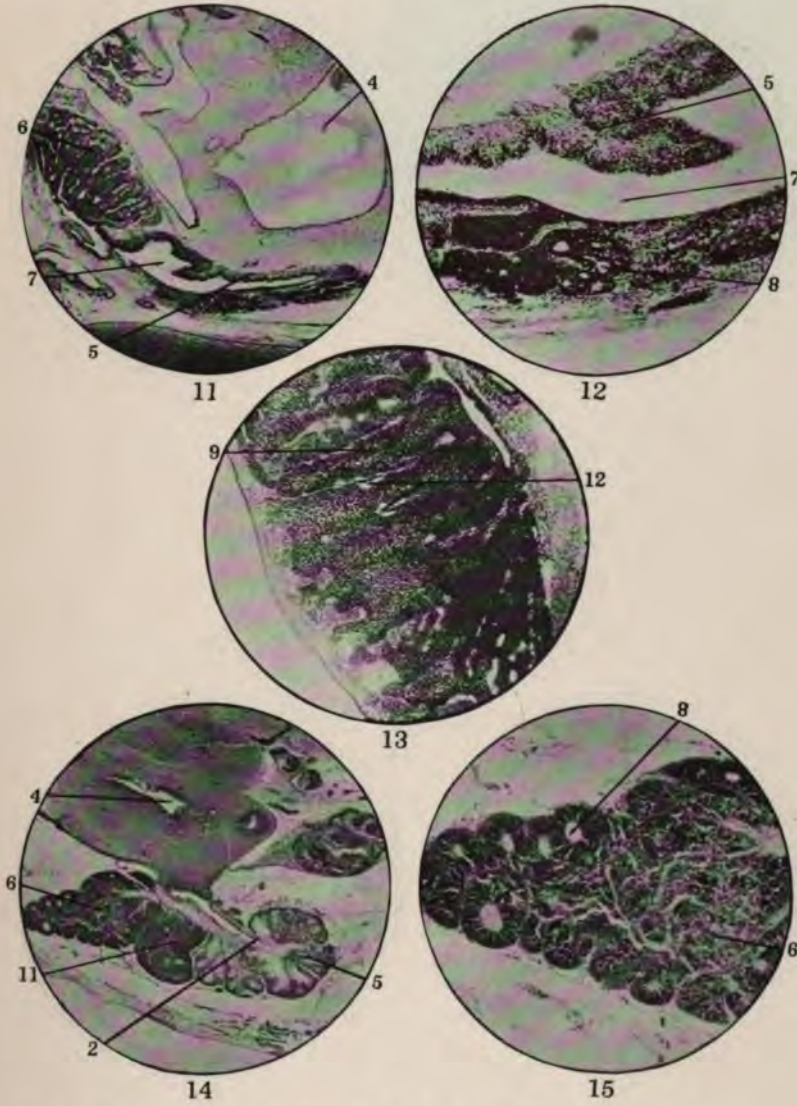


Fig. 11. *Squalus acanthias* entire gland. $\times 50$.
Fig. 12. *Squalus acanthias*, juxta-neural epithelial portion. $\times 100$.
Fig. 13. *Squalus acanthias*, distal epithelial portion. $\times 75$.
Fig. 14. *Anguilla vulgaris*. Entire gland. $\times 50$.
Fig. 15. *Anguilla vulgaris*. Distal epithelial portion. $\times 150$.

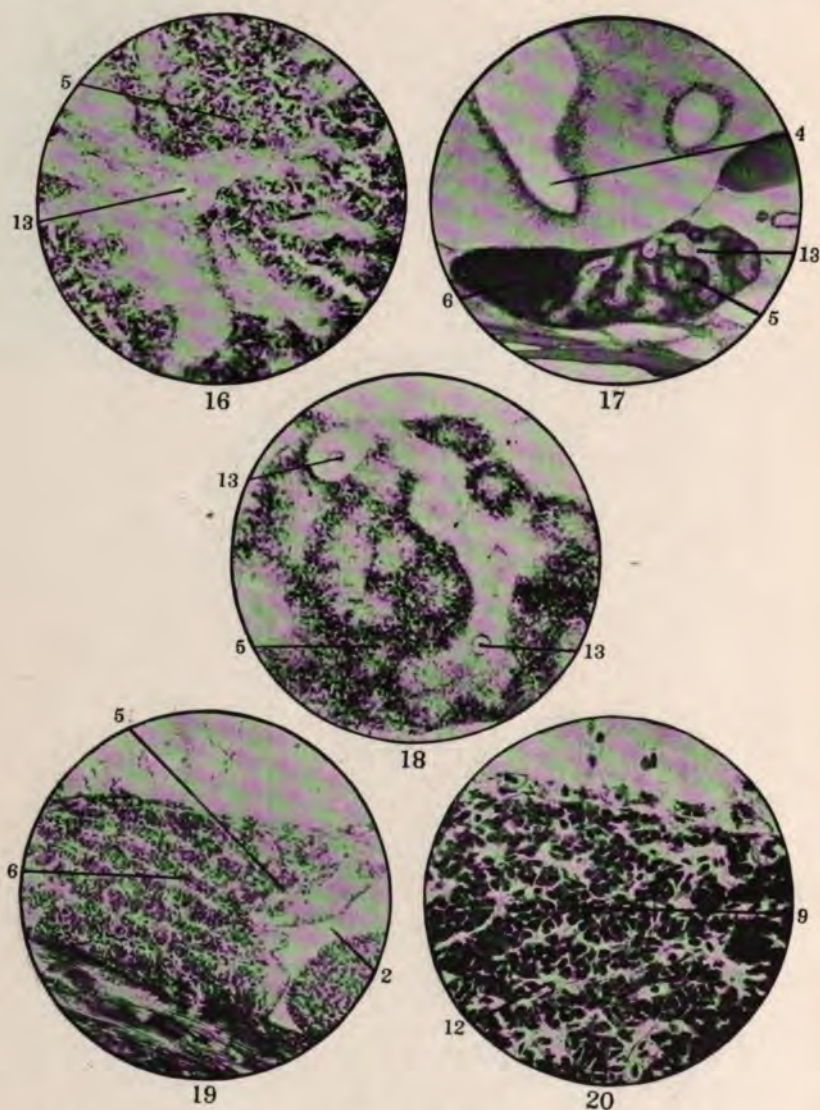


Fig. 16. *Anguilla vulgaris*, Juxta-neural epithelial portion. $\times 150$.
 Fig. 17. *Lepidosteus osseus*. Entire gland. $\times 35$.
 Fig. 18. *Lepidosteus osseus*. Juxta-neural epithelial portion. $\times 150$.
 Fig. 19. *Diemyletus viridescens*. Entire gland. $\times 115$.
 Fig. 20. *Diemyletus viridescens*. Distal epithelial portion. $\times 150$.

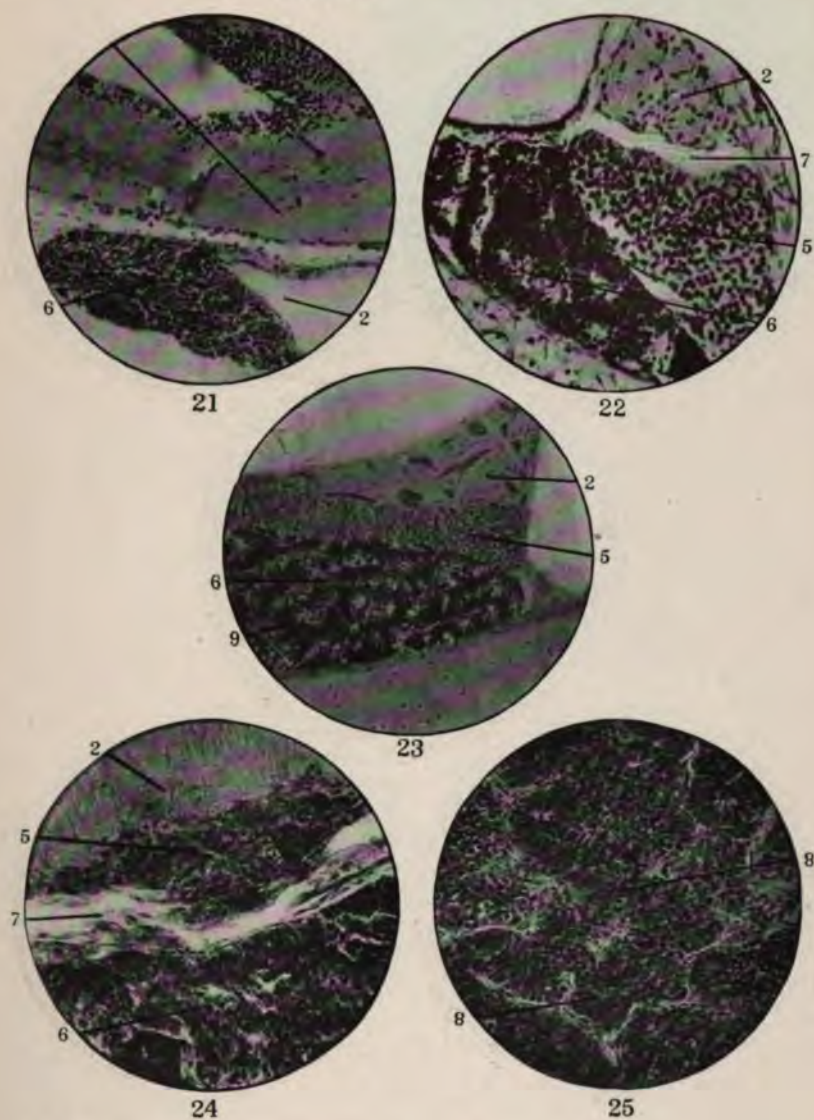
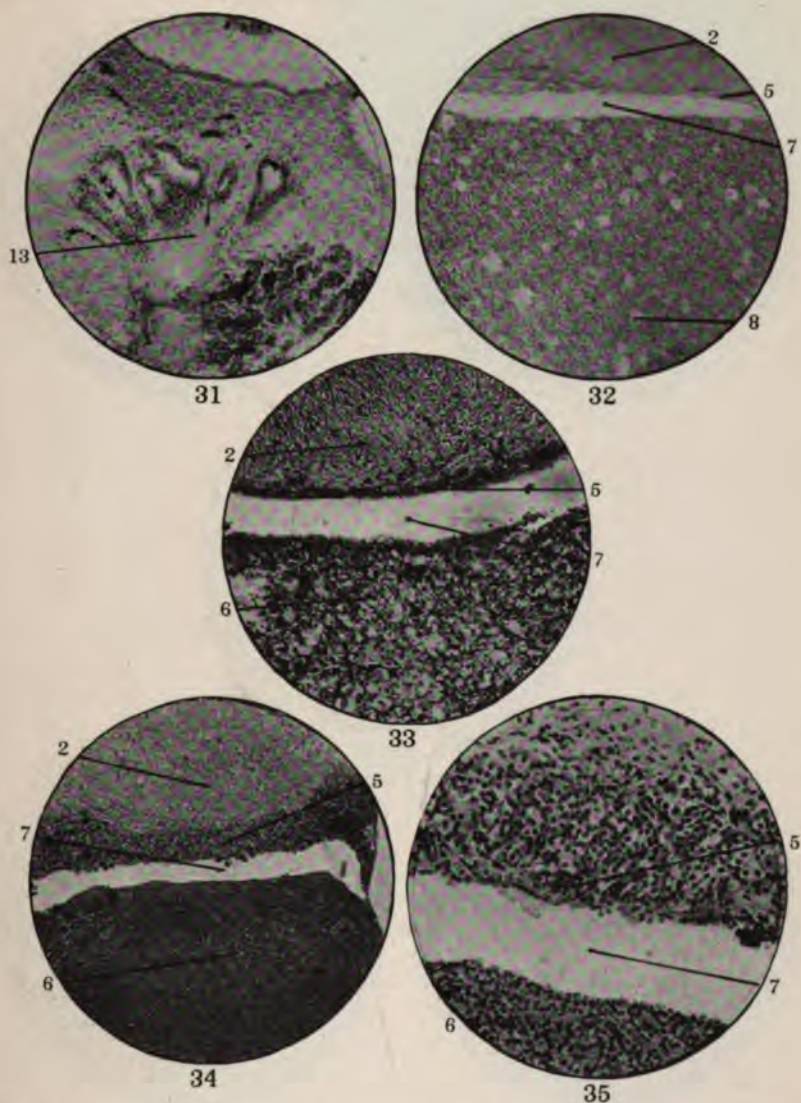


Fig. 21. *Menobranhus lateralis*. Entire gland. $\times 50$.
Fig. 22. *Rana sylvatica* (larval). Entire gland. $\times 125$.
Fig. 23. *Rana sylvatica* (adult). Entire gland. $\times 125$.
Fig. 24. *Cistuda carolina*. Three portions of body. $\times 75$.
Fig. 25. *Cistuda carolina*. Distal epithelial portion. $\times 150$.



- Fig. 26. *Eutaenia sirtalis*. Entire gland. $\times 35$.
 Fig. 27. *Alligator mississippiensis*. Three portions of body. $\times 90$.
 Fig. 28. *Alligator mississippiensis*. Branched tubular processus infundibuli. $\times 50$.
 Fig. 29. *Gallus domesticus*. Three portions of body. $\times 50$.
 Fig. 30. *Gallus domesticus*. Distal epithelial portion. $\times 150$.



- Fig. 31. Chick (last days of incubation). Branched tubular infundibular process. $\times 150$.
 Fig. 32. *Columba livia*. Three portions of the body. $\times 100$.
 Fig. 33. *Didelphys virginiana*. Three portions of body. $\times 70$.
 Fig. 34. *Mus decumanus*. Three portions of body. $\times 75$.
 Fig. 35. *Mus decumanus*. Three portions of body. $\times 150$.

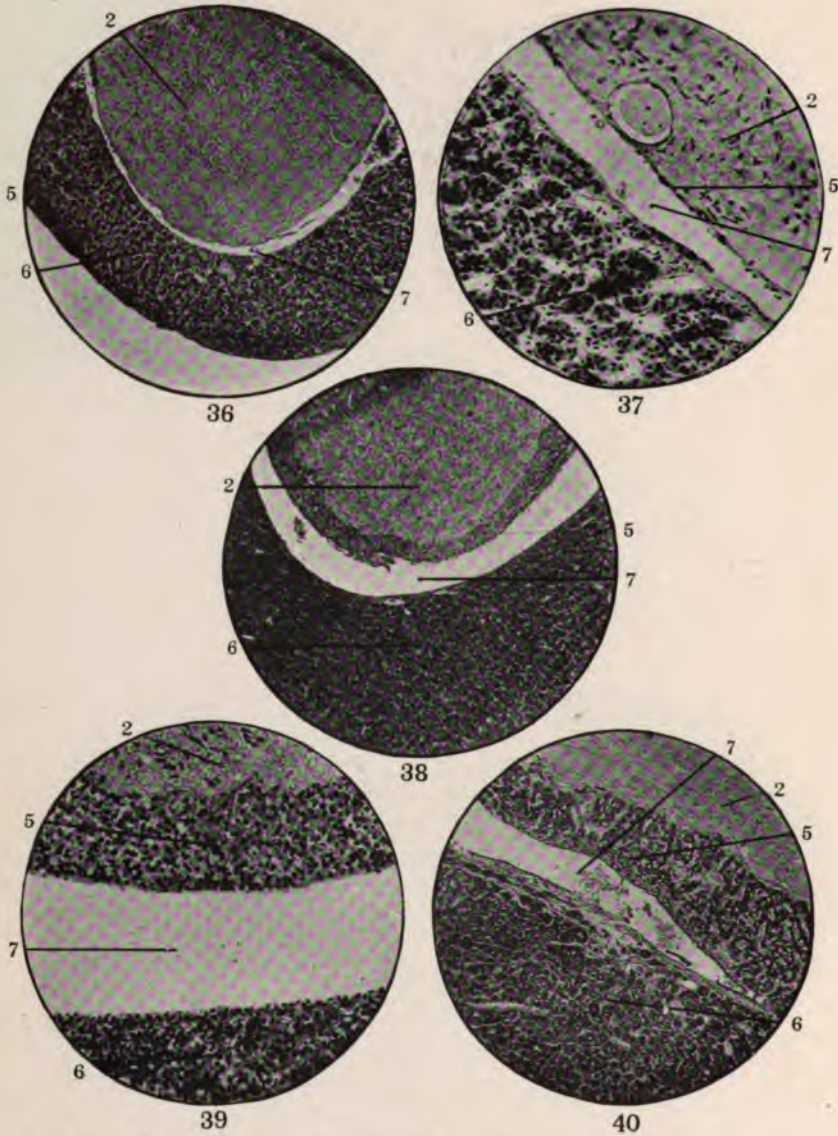


Fig. 36. *Felis domestica*. Three portions of body. $\times 75$.
 Fig. 37. *Felis domestica*. Three portions of body. $\times 150$.
 Fig. 38. *Canis familiaris*. Three portions of body. $\times 50$.
 Fig. 39. *Canis familiaris*. Three portions of body. $\times 150$.
 Fig. 40. *Ovis aries*. Three portions of body. $\times 75$.

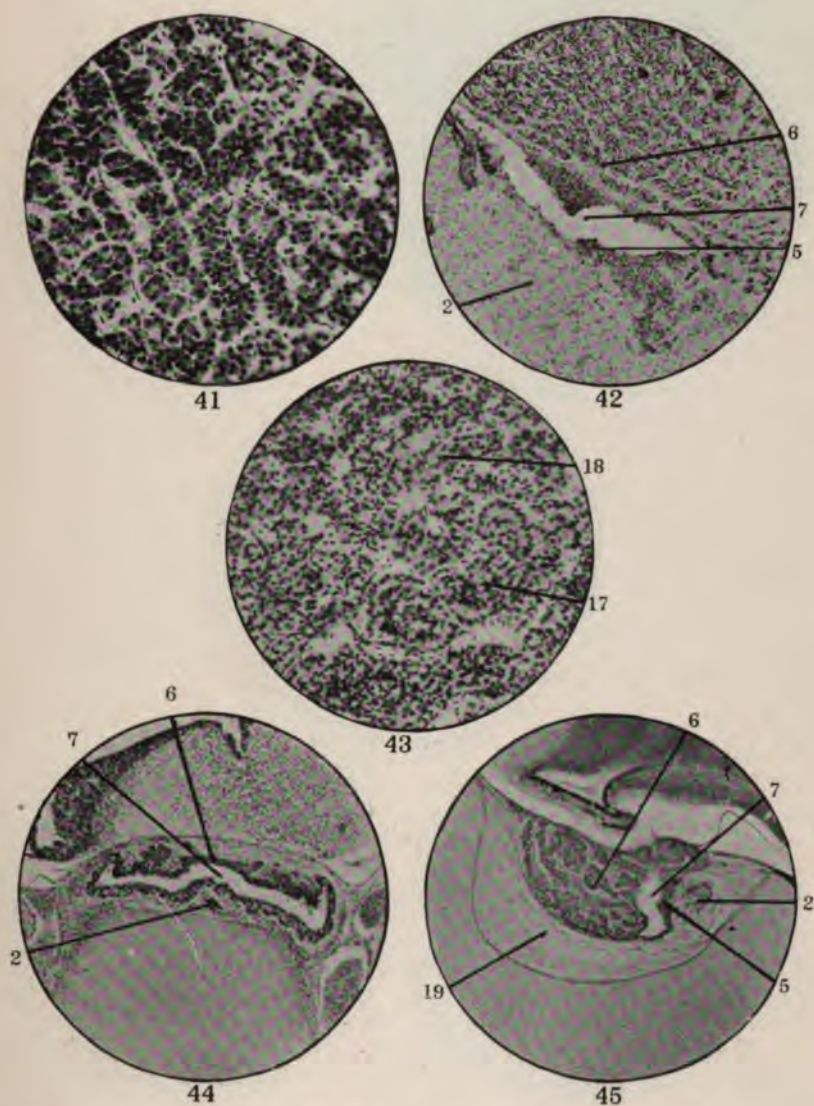


Fig. 41. *Ovis aries*. Distal epithelial portion. $\times 150$.
 Fig. 42. *Cynocephalus babuin*. Three portions of body. $\times 65$.
 Fig. 43. *Cynocephalus babuin*. Arrangement of basophiles. $\times 150$.
 Fig. 44. Human embryo 20 mm. Anlage of body. $\times 65$.
 Fig. 45. Ten week human fetus. Three portions of body. $\times 50$.

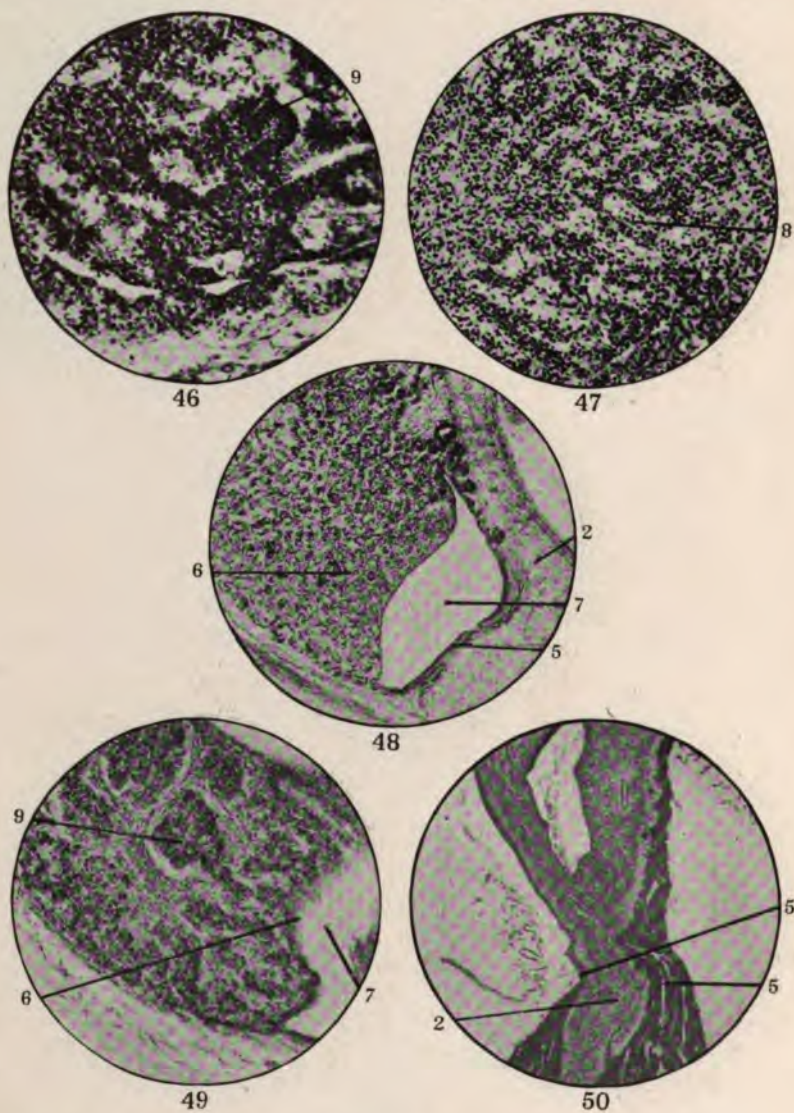


Fig. 46. Twelve week human fetus. $\times 65$.
Fig. 47. Fourteen week human fetus. $\times 75$.
Fig. 48. Twenty week human fetus. $\times 35$.
Fig. 49. Twenty-four week human fetus. $\times 50$.
Fig. 50. Twenty-eight week human fetus. $\times 35$.



Fig. 51. Thirty-two week human fetus. $\times 75$.

Fig. 52. Thirty-six week human fetus. $\times 35$.

Fig. 53. Eight month infant. $\times 50$.

Fig. 54. Eight month infant. $\times 100$.

Fig. 55. Human adult. Transverse. $\times 50$.

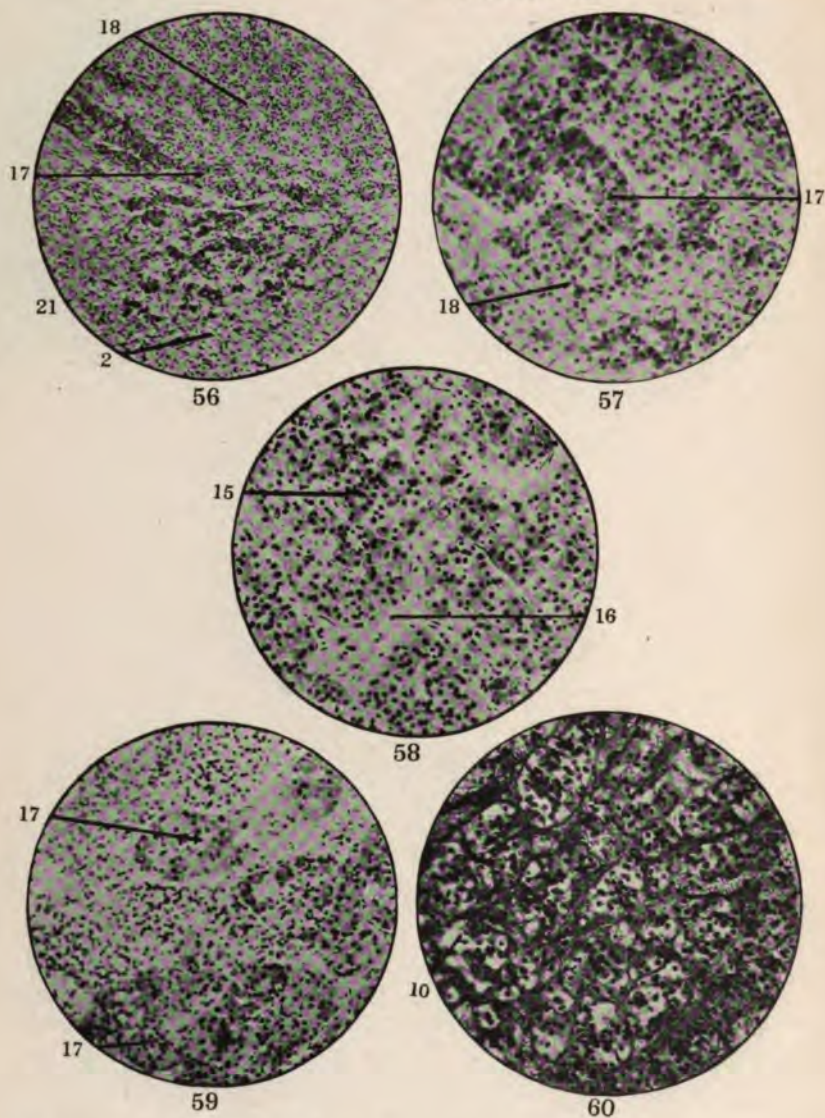


Fig. 56. Human adult. Transverse. $\times 75$.
 Fig. 57. Human adult. Transverse. $\times 150$.
 Fig. 58. Human adult. Transverse. $\times 150$.
 Fig. 59. Human adult. Transverse. $\times 150$.
 Fig. 60. Human adult. Transverse. $\times 150$. (Showing blood spaces among acidophiles).

FUS VAS	RESIDUAL LUMEN, ITS CHARACTER	BLOOD VASCULAR ELEMENTS	CONNECTIVE TISSUE	COLLOID MATERIAL
ent. Sit teriorly	None observed.	Between cords and columns.	Absent.	Absent.
ent. Sit teriorly.	Present. Large and with several diverticula.	In distal portion only between columns.	Absent.	Present in saccus.
ent. Sit teriorly	None observed.	In distal portion.	Present in distal portion.	None observed.
ent. Sit teriorly	None observed.	In distal portion only.	Present in distal portion.	None observed.
ent. Sit teriorly	None observed.	In distal portion and along border of juxta-neural portion.	None.	None.
ent. Sit teriorly	None observed.	In distal portion.	None.	None.
ent. Sit teriorly	None observed.	In distal portion.	None.	None.
ent. Sit teriorly	Present, situated above.	In distal portion.	Fine frame-work.	In some acini.
ent. Sit teriorly	Present, situated above and in front.	Offshoots from saccus vasculosus.	Fine frame-work.	None.
ent. Sit teriorly	Present, situated above and behind.	Offshoots from saccus vasculosus.	Fine net-work.	In some acini.
ent. Sit teriorly	Present. Situated above.	Rich plexus in distal portion.	Fine frame-work.	In some acini.
ent. Sit teriorly	Present. Situated above. Thin posterior wall. Thick anterior wall.	Rich plexus in distal portion.	Fine net-work.	In some acini.
ent. Sit teriorly	Present. Situated above and behind. Relatively thick posterior wall. Anterior wall thin.	Rich plexus in distal portion.	Fine net-work.	In many acini.
ent. Sit teriorly	Present. Situated above. Thick anterior and posterior walls.	Rich plexus in distal portion.	Fine net-work.	In many acini.
ent. Sit teriorly	Present. Situated behind. Posterior wall thick. Anterior wall, the distal portion.	Rich plexus in distal portion.	Distinct lobulation.	In many acini and lumen.
ent. Sit teriorly	Present. Situated behind. Thin posterior wall. Thicker anterior wall.	Rich plexus in distal portion.	Distinct frame-work.	In many acini.
ent. Sit teriorly	Present. Situated above and behind. Thick anterior and posterior walls.	Rich in distal portion.	Distinct frame-work.	In many acini.
ent. Sit teriorly	Present. Irregular in outline. Both walls faintly staining basophiles. Walls not very vascular. Posterior wall narrow.	Especially rich vascularisation among faintly staining basophiles.	Distinct tendency toward lobulation among faintly staining acidophiles.	None observed.
ent. Sit teriorly	Well marked transverse slit.	Absent.	Absent.	Absent.
ent. Sit teriorly	Wide. Situated posteriorly. Thick anterior and posterior wall.	None.	None.	None.
ent. Sit teriorly	Wide, situated posteriorly. Thick anterior and posterior wall.	None.	None.	None.
ent. Sit teriorly	Wide. Situated posteriorly. Thick anterior and posterior walls.	A few spaces.	Some strands just entering.	None.
ent. Sit teriorly	Present. Situated posteriorly. Thick walls.	Many wide spaces in distal portion.	Rich trabeculation.	In some acini.
ent. Sit teriorly	Wide. Situated posteriorly. Thick anterior and posterior walls.	Many wide spaces in distal portion.	Rich trabeculation.	None.
ent. Sit teriorly	Present. Situated posteriorly. Posterior wall reduced in thickness.	Rich plexus in distal portion, especially in acidophilic areas.	Rich trabeculation.	In acini and lumen.
ent. Sit teriorly	Greatly reduced. Thin anterior wall. Irregular posterior wall. Accessory pockets.	Rich plexus in distal portion.	Rich trabeculation.	In acini, lumen and accessory pockets.

MEMOIRS
OF
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
No. 3

EARLY STAGES OF VASCULOGENESIS
IN THE CAT (*FELIS DOMESTICA*)
WITH ESPECIAL REFERENCE TO
THE MESENCHYMAL ORIGIN
OF ENDOTHELIUM

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FROM THE ANATOMICAL LABORATORY OF COLUMBIA UNIVERSITY

PHILADELPHIA, PA.
1914

VASCULOGENESIS IN THE CAT

Of the many problems that engage the attention of students of the vascular system, none is more fundamental, and hardly any more perplexing and confused today, than that of the origin of the simple squamous epithelium (endothelium) which is the common lining of all vascular channels, whether haemal or lymphatic. It has been held, and the opinion of late has been zealously propagated, that so far as the vessels of the body are concerned, their endothelium arises solely from the already formed endothelium of the extra-embryonic vessels, or if there is any production of endothelium within the body of the embryo, it is for so brief a period and so inconsiderable in amount as to be theoretically negligible.¹ For the supporters of this view Rabl's dictum, "Endothel stammt nur von Endothel" is still a satisfactory summary of the facts. A logical sequence of this opinion is the doctrine of the specificity of endothelium, obviously a much broader generalization, which takes endothelium out of the series of mesenchyme derivatives and separates it absolutely from the blood, should that prove to be of mesodermal origin.¹ Consequent thinkers who support this view have seen the importance of assigning an entodermal origin to both blood and endothelium. Could this be established, it would lend a degree of antecedent probability to the doctrine of the angioblast but, strictly speaking, it is not a necessary postulate, for a tissue arising diffusely from the mesenchyme, may as development proceeds, be confined to definite localities or even ultimately be restricted to homoplastic proliferation. Such a tissue would be specific if it yielded no heterogeneous products, and I feel myself that this latter property enters more largely

¹ Minot, Evans, and Sabin in Keibel and Mall, *Handbuch d. Entwicklungs-gesch. d. Menschen*, Leipzig, 1912. These writers have made a clear and emphatic statement of the doctrines of the Angioblast and of the specificity of endothelium, and presented the evidence which may be adduced in favor of these views very completely. That they have not considered all the available evidence, however, is apparent from their all but complete omission of the results of recent haematology (since 1909), which would indicate the wisdom of some reserve in accepting as final generalizations which neglect such extensive and important data.

into our idea of specificity as applied to tissues than the community of origin of their elements. It would seem, therefore, that the advocates of specificity have charged themselves rather to prove that endothelium, once formed, produces only endothelium and never any other elements, as say blood or connective tissue, than that it is peculiar in its origin.

On the other hand, it has been held that endothelium is simply a derivative of the mesenchyme from which it arises diffusely by a multitude of separate anlagen. This view does not undertake a general interpretation of the mesoderm and mesenchyme and the apparent variations of their mode of origin as determined by the yolk-content of the ovum, but merely states that endothelium arises out of elements which have once formed a part of the general mesenchyme complex. A development of this point of view is the adaptive theory of endothelium, which holds that endothelium is a modification of mesenchyme, and by no means a stable one at that, but owes its presence to hydrodynamic forces, and when these cease to operate, endothelium disappears as endothelium and reverts to mesenchyme, or gives rise to products indistinguishable from those of mesenchyme. Endothelium is taken to be a form which mesenchyme assumes in certain positions which can not be retained with the loss of position and is then analogous to the biometamorphoses of plants grown under unnatural conditions, which are lost when they are returned to their normal environment.

It is conceivable that some supporters of the adaptive theory of the origin of endothelium may not feel themselves obliged to maintain a variety of products on the part of endothelium, just as it is not to be required of those who hold the specific doctrine that they should demonstrate an entodermal origin. The former might then hold endothelium to be a derivative rather than a modification of mesenchyme; it would satisfy them to show the multitude of its separate anlagen, their wide diffusion through the mesenchyme, in the somatopleure as well as in the splanchnopleure, within the body of the embryo as well as in the yolk-sac, and their continued formation in the embryo during an appreciable period of development, and along the major

vascular lines. This done, they could allow endothelium to manifest such specificity as it might acquire, as in the case of other mesenchymal derivatives, cartilage for example, or cross-striated muscle, which increase by accretion of new elements as well as by the proliferation of those already formed, and yet once formed, are specific to the degree that they probably do not give rise to other types of connective tissue.

In thus comparing the angioblastic with the mesenchymal, the specific with the adaptive views of endothelium, I have alluded to certain differences of opinion regarding the supposed mode of its wide dissemination through the connective tissue of the body, which are fundamental and may now be contrasted. The doctrine of specificity holds this process to be essentially one of growth. The period of development of endothelium is amazingly short, and the site, for most entertainers of this view, is the yolk-sac at some distance from the embryo. For others there is an abstract possibility that certain parts of certain embryonic vessels may develop *in situ*, but it is not unfair to say that they consistently tend to minimize and curtail the developmental period of endothelium. The important part of their doctrine, the part which has engaged their chief attention, and is presented with most conviction, is the growth of the endothelium by solid sprouts from the ends of hollow injectible vessels. This is the sole source of endothelium (the case of a few embryonic vessels apart), and so far as I am aware, absolutely the only evidence of its specificity.

The adaptive theory finds support in the presence, through a considerable period of ontogeny, of numerous separate sacs or vesicles of endothelium, definitely localized in positions subsequently occupied by continuous vessels, and in the gradual enlargement and ultimate fusion of these vesicles to form the vessels. Further, it notes that the vesicles are preceded by minute clefts or spaces in the mesenchyme bounded by ordinary mesenchyme cells, which subsequently flatten and eventually form a continuous layer as the vesicles enlarge. This view thus extends the period of development, and enlarges the area in which it is active to practically the whole body of the embryo. It does not

deny that endothelial cells undergo mitosis, that vessels after formation lengthen, or that they may branch and send out sprouts; for it by no means implies that endothelium does not grow, but it relegates these phenomena to a later period in the life of the embryo, and endeavors to distinguish between a cytomorphosis terminating in a definable type of cell (development, genesis, anlage, das Werden) and a proliferation and extension of a fully formed tissue (growth, das Wachsen).

One further point, the great vascularity of the embryo as compared with the adult makes the fate of the temporary vessels and their endothelium of some importance to a general theory of this tissue. The adaptive view considers that they revert to mesenchyme, the doctrine of specificity that they disappear.

Divergent principles of interpretation rather than differences in method and technique underlie, I believe, these discrepant theories, although I am aware that the contrary has been emphatically proclaimed, and in particular that the method of injection has been held to be the ultimate criterion in these matters. Without discussion at this point of what I believe to be the claims of enthusiasm, it is still possible to admit the objective reality of the positive data obtained by this method, without attaching finality to its negations. One may admit that where the injection passes, there is a channel, without denying that beyond the limit of injection there are forming channels, or considering it proved that injected channels are always lined by a complete endothelium. One may admit that the blind terminals of vessels are actual terminals, without assuming that they are always sprouts, for they may be regressive. And above all, one may admit the frequency of extravasation, without holding it to be *ohne weiteres* an artifact. But if these reservations are allowed, or even conceded to be still matters of free and unbiased inquiry, the positive results of the injection method will form but a slender basis for such imposing doctrines as those of the specificity of endothelium, and of the angioblast.

From the time of the establishment of the circulation, the body of the embryo and some of its membranes are supplied with continuous channels which distribute the circulating medium, and

return it to the heart. From this period throughout life, such continuous vessels are present and functionally active. In the adult body it has long been customary to inject them for convenience in demonstrating their course. In the foetus also, such procedures are familiar and have been held to demonstrate the plan of organization of the circulatory channels at the period of examination. Now it is obvious that the injection at earlier periods, even in young embryos, is a fact of the same order, however admirable the technique or delicate the procedure—it simply demonstrates continuous channels, which it may be presumed are functionally active. It reveals the extent and conformation of the formed and functional vascular system and enables the observer to follow its successive stages from the period at which injection first becomes practicable. In such studies, however, injection has no monopoly, but it shares its data with the method of direct observation of living tissue when the object is small and transparent, and with the exact but laborious method of reconstruction. On the other hand, it is idle to pretend that the injection method throws any light on areas beyond the limit of the actual injection, or affords the slightest presumptive evidence of their histological condition, in particular, as regards the presence or absence of small discrete vesicles of endothelium.

The dilemma is simple; we may recognize or refuse to recognize the uninjectible periphery. If we recognize it, the method of injection becomes not only partial and incomplete, but subordinate to the methods which reveal all of its findings and in addition enlarge our field of observation. It ceases to be a critical method. If we refuse to recognize the periphery beyond our injection, we have begged the question at issue, our vessels are growing not only in a foreign tissue, as the angioblast doctrine believes, but in a theoretical vacuum and the only possible source of increment is the proliferation of their own elements. We reach the only conclusion the exclusive study of organized vessels by any method would allow, the formation of the new out of the old by sprouting. A comparison may perhaps serve to illustrate the inconvenience of taking continuity of lumen as the critical index of structure. Had the metanephros chanced to

be first studied by the method of injection, we should have a history of the ureteric bud from the standpoint of its lumen. It would have sprouted, giving branches of many orders, and by growth in continuity its arborizations would in time form their definitive terminals, the Bowman's capsules. Should an investigator, with a belated confidence in slides and reconstruction, have ventured to observe the renal vesicles, he might have been told he had been misled by artifacts. Had he objected that they were constant, had definite structural characters, and were localized with exquisite precision, he might at length have obtained their tardy recognition. But it is probable, for doctrines as well as masses have momentum and inertia, that he would then have been informed, that his vesicles were but separated sprouts, because nephrothelium comes only from nephrothelium. And these arguments would be as justifiable, nay as necessary, in the one case as in the other, if ultimate reliance is placed on the injection method which can only reveal continuity of lumina. The logic is good, the argument the typical scholastic deduction of the special case from the general principle, but the principle is intuitive and arbitrary, not inductive.

The blind terminals of vessels in the mammalian foetus are a favorite topic for study by injection, but so far as I am aware, the possibility of their regressive nature has never been seriously considered; it has simply been assumed that they were always growing sprouts. I desire here rather to note the gap in the evidence than to question this mode of growth as a factor in the extension of formed vessels in late embryonic stages and in the foetus.

W. G. MacCallum² ('02) made a careful and excellent histological study of lymphatic terminals in the skin of pig foetuses, from 6 cm. to 15 cm. in length, and reached the conclusion that the lymphatics did not communicate with tissue spaces. From their extremities project strands of cells in a single or double row which run for some distance in the embryonal connective tissue, or join another lymphatic. He thought that few of these cells were in syncytial relation with adjacent fibroblasts.³ Into the

² 1902. Arch. f. Anat. u. Entwicklungsgesch., p. 273.

³ Exponents of the doctrine of specificity seem inclined to lay stress on this point as indicating the independence of endothelium. In view of the interdermal

bases of the cords, the injection entered in a thin stream for a short distance revealing a cleftlike diminishing lumen.

Bartels ('09)⁴ has given figures which closely correspond to those of MacCallum, but he considers the interpretation of the cell-cords as sprouts debatable. On the other hand, Miss Sabin's figure of lymphatic terminals in a 5 cm. pig ('02, fig. 2)⁵ represents very different conditions; here are blind, blunt terminals and no sprouts. She states, however, that occasionally from one of these blunt ends, the "injecting fluid can be forced out into a long thread-like process" which is a sprout and represents the process of growth.

She has plainly described a somewhat different structure from the solid uninjectible strands of MacCallum and Bartels, though not improbably a phase of the same process. A number of interpretations suggest themselves:

1. Miss Sabin's, that the slender injectible processes are sprouts growing from formed vessels and that this is the only mode of their extension at this period.

2. That in view of their infrequency, we are dealing with the inception of autonomy on the part of the endothelium, which in a few places is sprouting, while in most it is advancing by accretion, as has been definitely proved for the thoracic duct and the systemic lymphatics in general in embryos.⁶

cytodesmata described by v. Szily, Studnička and others, it is of course void of theoretical importance in this sense, for if in general, elements of different germ layers may stand in syncytial relation to one another, the specificity of endothelium is in no way impaired by a like continuity with the mesenchyme, which in fact it does possess in the embryo. If in the foetus a separation does actually occur, it ought to be interpreted in the light of the general tendency of the organism to resolve its synetia into independent cells, and not as a peculiar sign of the specificity of endothelium.

⁴ 1909. *Das Lymphgefäßsystem*. Jena. p. 12.

⁵ 1902, *Am. Jour. Anat.* vol. 1, p. 367.

⁶ Huntington & McClure, 1907, *Am. Journ. of Anat.*, vol. 6, *Abstr. Anat. Rec.*, vol. 1, pp. 36-41. Huntington, 1908, *Anat. Rec.* vol. 2, pp. 19-45; 1910, *Anat. Rec.*, vol. 4, pp. 339-423; *Compte Rendu 16 Congrès Internat. de Med. Sect. 1*, fasc. 2, pp. 127-142; *Anat. Anz. Ergän. z. Bd. 37*, pp. 76-94; 1911, *Wistar Mem. No. 1*; *Anat. Rec.*, vol. 5, pp. 261-276. Stromsen, 1912, *Anat. Rec.*, vol. 6, pp. 343-356. Kampmeyer, 1912, *Am. Jour. Anat.*, vol. 13, p. 401, and *Anat. Rec.*, vol. 6, p. 223; McClure, 1912, *Anat. Rec.*, vol. 6, p. 233. Tilney, 1912, *Amer.*

3. That the sprouts are regressive and that here, as in other tissues (bone, striated muscle), progressive and retrogressive processes co-exist.

4. That the 'sprouts' are the last stages in the annexation of vasoformative cells which have built themselves into cords, joined the lymphatics and are now establishing their lumen. Only in this last stage would they be accessible to injection. This interpretation is suggested by Bartels ('09) and is of considerable import. Referring to certain unicellular sprouts of MacCallum, he says:

Gerade, dass es einzelne Zellen sind, welche die Stränge bilden, scheint mir eher für den Modus der Aufreihung dieser Zellen durch den zentralwärts gerichteten Saftstrom als für eine zentrifugal gerichtete Sprossung zu sprechen. Doch will ich darauf, als auf eine nach dem bisher Vorliegenden nicht sicher zu entscheidende Sache, ebensowenig eingehen, wie auf die Beurteilung des Verhaltens der benachbarten Gewebsbestandteile zu den Gefässen. Beides scheint mir weiterer eingehender Untersuchungen zu bedürfen.⁷

None of these observations, important as are the data they have collected, have given us the means of interpretation, once the increment of vessels is conceived as a minute process which must be treated in terms of cells and cell modifications. For this it is necessary to work in confined areas of well understood topography and with a minutely graded and continuous series of embryos. If cords of single or double rows of cells are to be studied developmentally, we must at least give ourselves the chance of seeing cell add itself to cell, or the sprout advance from the unicellular to the multicellular condition, and not work at wide intervals of time or with material from various regions where only gross comparisons are possible and minute processes may altogether escape us.

From the standpoint of possible retrogression, the work of Spuler⁸ and of Fuchs⁹ is interesting and their aloofness from this particular problem gives their testimony additional importance.

Journ. of Anat., vol. 13, pp. 193-220. Miller, A. M., 1913, Amer. Journ. of Anat., vol. 15, pp. 131-163. Allen, W. F., 1913, Quart. Journ. Micr. Sci., vol. 59, pp. 309-360.

⁷ Loc. cit., p. 45.

⁸ 1892, Arch. f. mikr. Anat., Bd. 40, p. 530.

⁹ 1903, Anat. Hefte. Band. 22, p. 97.

They were writing in refutation of Schaffer's derivation of erythrocytes from vasoformative cells and of Minot's¹⁰ expansion of this observation into an absolute distinction between nucleated and non-nucleated red corpuscles, and his classification of blood in the vertebrates on this basis.

Spuler showed clearly that the erythrocytes in question and the vasoformative cells containing them were degenerative. Fuchs, working with the omentum of the guinea pig in the first few days after birth, confirmed these observations. The vasoformative cells of Schaffer¹¹ and Ranvier¹² he found usually connected with functional vessels by narrow cell-strands, with or without lumina. In a few cases the strands were interrupted. This he attributed to rupture occasioned by unequal growth in the omentum. He subscribes to the production of vessels from vasoformative cells but makes a distinction between these cells and the erythrocyte containing cells which he found, as Spuler had done before him, usually connected with vessels. These, in view of their degenerating contents, may fairly be deemed retrogressive. Their occasional occurrence free in the connective tissue then becomes a late and not an early phase in their history. The order of events in a degenerating capillary would appear to be a collapse of its lumen at some intermediate point or points, between or beyond which the vessel and content persist for a time; the solidification and the reduction of the collapsed regions to double or single rows of cells and eventually long protoplasmic strands, which finally give way, and the remnants of lumina with their contents of blood cells and their wall of endothelium become isolated in the connective tissue. Fuch's excellent illustrations are astonishingly like the sprouts of MacCallum and the cell cords of Bartels, differing only in the cellular contents of the vessels (cf. Fuchs, fig. 2, with Bartel's '09, fig. 12, and Fuch's, figs. 4, and 3, with MacCallum, figs. 6 and 7, respectively).

Identical appearances are observed in definite regions of younger embryos of the cat incident to vascular processes which are

¹⁰ 1890, *Anat. Anz.*, Bd. 5, p. 801. ¹¹ 1874, *Mo. Mic. Journ.*, vol. 11, p. 97.

¹² 1874, *Arch. de Physiol.*

known to be regressive, because they are preceded by blood containing plexuses and functional vessels and accompany their resolution. Huntington¹³ has noted the condition in connection with the perivenous plexuses:

Some of the elements develop into permanent tributaries of the main veins. Others undergo a process of separation from the permanent functional channels and degenerate. In many cases their blood cell contents break down and are eliminated, while their endothelial lining appears to revert to the indifferent type of the embryonic mesodermal cell.

Thus in embryos between 13.5 and 16 mm. many striking instances of this reversion are to be observed. [And further] these detached and retrograding channels constitute lines around which the most active primary lymphatic organization of the mammalian embryo centers.

I am far from wishing to claim that 'sprouts' are never growing points, I would only instance cases where they certainly are regressive, and express the doubt that their injectibility or lack of it affords a proof of their origin from endothelium. The subject is a promising one and would repay unprejudiced observation under conditions where the direction of the process might be definitely ascertained—in a circumscribed area and with a closely graded series of foetuses. It may be that their formation in the lymphatic system will prove to be a late phenomenon and incident to the ultimate sealing off of the lymphatics from the tissue spaces, if a closed system of lymphatic capillaries actually exists.

The question of extravasation is perhaps the most difficult of the many problems the injection method has raised, and is giving us a literature of as much subtility in connection with developing vessels as it has done in regard to the circulation in the adult spleen. The point at issue is not the permeability of the vessel wall which may be taken as proved beyond controversy by the facts of metabolism, and the passage of erythrocytes and leucocytes, as well as by the results of injection. The real difficulty is as to how we may accept these indisputable facts and yet maintain that when extravasations occur during injection they prove a discontinuity between the lumen of the vessel and the tissue space in mesenchyme or in connective tissue.

¹³ 1911, Memoirs of the Wistar Inst. of Anat. and Biol. No. 1., Philadelphia.

The positive conclusion that extravasation means communication between vessel and tissue space seems justified when the injection is controlled by a manometer and practised at physiological or subnormal pressure, as in Herring and Simpson's¹⁴ injections of the liver or Pustoiwoitow's¹⁵ of the spleen. If, as in these cases, the injected substances escape under less than normal pressure it is permissible to infer a real continuity, though strictly speaking it is necessary to confirm this by direct observation of the tissue (*vide infra*).

But if the pressure is not thus controlled, it will always be possible to interpret the extravasation as an artifact, for it can always be argued that rupture occurred under excessive pressure. Centrifugal injections of lymphatics are obviously subject to this doubt, so that the presence here of extravasation is only suggestive and not demonstrative of communication between these terminals and the tissue spaces.

Other methods of control than that of manometric measurement of the pressure can not be freed from subjectivity. To observe, for example, the passage of the injection with a binocular and discontinue when it begins to extravasate is arbitrary; to succeed in stopping at the moment when extravasation has just begun, when the terminals have still but a mossy appearance, shows only the point to which the observer's dexterity may be developed. One may, with practice, heat a thermometer rapidly till the mercury rises to a chosen point, and become able to discontinue just as the point is reached. The repetition of the act may increase exactitude, but will not prove the absence of luminal continuity beyond, however we might argue, when we had just passed our selected point, that we had ruptured a delicate and invisible membrane.

Nor is even the "explosive" (MacCallum) rupture of the vessel wall at some intermediate portion of its course a proof that its terminals are closed. A very moderate experience with the injection of adult material will convince anyone that large veins may rupture before their radicles are filled, and the rupture may

¹⁴ 1906, Proc. Roy. Soc. B., vol. 78.

¹⁵ Arch. f. Anat. u. Entwicklungsgesch., p. 219.

be attended with sensations, even emotions, on the part of the observer. Yet it cannot thereby be proved that the vessels end blindly; and we ought not to assume, because of the delicacy of embryonic structures that their physical properties are different in kind, or obey other general laws of stress and strain than larger tubes which are more accessible to examination.

But while the results of injection demonstrate the permeability of the vessel wall, as do in life the passage of cells and fluids, they do not afford us the opportunity of ascertaining the structure of the wall or the nature of its orifices. They simply give us the concept of permeability, which it is not permissible, without further grounds, to translate into porosity. Every fact of extravasation can be admitted and the integrity of a completely closed vessel wall still be maintained if we introduce the concept of a structureless (invisible) membrane, the resistance of which to pressure is practically *nil*.

Helly¹⁶ has shown this with admirable clearness in his interesting work upon the vessels of the spleen. He confirmed previous observations of the passage of injections through the vessel walls, whether solutions, suspensions, colloidal masses, or foreign blood corpuscles were used. He further washed out the red blood corpuscles from the pulp spaces by saline irrigation, and caused their numbers in the pulp to increase by retrograde injection (obstruction of the splenic vein) and finally in sections of $5\ \mu$ observed both erythrocytes and leucocytes in passage through the walls of the sinuses. Yet he considered the wall complete.

Was zunächst den Widerstand anlangt, welchen die Gefässwand dem Durchtritte fester und flüssiger Bestandteile entgegenzusetzen vermag, so ist ersichtlich, dass derselbe bei den venösen Capillaren nur sehr gering, an gewissen Stellen der Wand überhaupt fast gleich null ist; gleicht sie doch, von der Fläche betrachtet, sehr einem Gitter, dessen Lücken vielfach gross genug sind, um ein rotes Blutkörperchen ohne jede merkliche Formveränderung durchtreten zu lassen. Dem zwischen beiden Bestandteilen des Gitters,—den inneren, parallel zur Längsachse des Gefässes angeordneten, stabförmigen Endothelzellen und den äusseren, quer um dasselbe verlaufenden Kreisfasern,—befindliche unmessbar dünnen strukturlose Häutchen kann wohl kein irgend nennenswerter Einfluss im Sinne einer Behinderung der Diapedese zugeschrieben werden und dies umso weniger, als das gedachte Häutchen sehr hinfällig ist

¹⁶ 1903, Arch. f. mikr. Anat., Bd. 61, p. 245.

und ungemein leicht zerstört wird. (p. 292) Die Milz hat ein, überall von einer regelmässigen Endothelschichte ausgekleidetes, daher geschlossenes Gefässsystem mit sehr durchlässigen Wandungen.

Den ich habe einerseits gezeigt, dass man unter Anwendung geeigneter Methoden sich davon überzeugen kann, dass der kreisende Blutstrom überall seinen Weg durch Capillargefässe nimmt, deren wand keine Oeffnungen in Gestalt beständiger Lücken für den Durchtritt rother Blutkörperchen zeigt, welcher dort, wo er der unmittelbaren Beobachtung zugänglich ist, als wahre Diapedese aufgefasst werden muss; andererseits habe ich den Durchtritt von Leukozyten durch die geschlossenen und unversehrten Gefässwände hindurch als thatsächlich vorhandene Erscheinung nachgewiesen.¹⁷

It will thus be seen that Mall¹⁸ fails to appreciate the subtilty of the question and at the same time misrepresents Helly in citing him as a believer in pores in spite of his emphatic statement to the contrary:

. . . recent authors are of one opinion regarding the large pores in the capillary walls. It appears that these openings are smallest according to Thoma, larger according to Helly, still larger according to Mall, and so large that they communicate most freely with the pulp-spaces according to Weidenreich.

Helly very carefully distinguishes between permeability and porosity, refuses to deduce the details of structure from physical and physiological properties, and accepts the structureless membrane of v. Ebner because he believes he has seen it. Mollier¹⁹ correctly represents Helly's position and criticises it with acumen:

Ob es freilich noch praktischen Zweck hat, von einer geschlossenen Röhre zu sprechen, wenn sie fast ohne Widerstand alle zelligen Elemente in unbegrenzter Menge hindurchlässt, könnte zweifelhaft erscheinen. Weidenreich hält jedenfalls eine solche Annahme für wertlos, wie ich aus seiner temperamentvollen Äusserung entnehme: 'Ein Sieb ist doch kein Topf.'

Ich meine aber, er vergisst hier, dass ein wesentlicher Teil unserer Vorstellung über den Vorgang der Diapedese der ist, dass Zellen durch die Capillarwand den Kreislauf verlassen, ohne dass dabei nennenswerte Mengen des Plasmas mit austreten. Der Kreislauf der Blutflüssigkeit wird also kaum beeinflusst.

Von diesem Gesichtspunkt aus hat die Annahme Hellys nicht nur ihre Berechtigung, sondern auch einen ganz bestimmten technischen Wert. Ob die Bezeichnung Diapedese auch für den Durchtritt roter

¹⁷ 1902, Arch. f. mikr. Anat., Bd. 59, p. 93.

¹⁸ 1903, Am. Jour. Anat., vol. 2, p. 315.

¹⁹ 1911, Arch. mikr. Anat., Bd. 76, p. 608.

Blutkörperchen ohne lokomotorische Eigenbewegung gebraucht werden kann, darüber will ich hier nicht weiter sprechen. Wir haben bloss damit zu rechnen, dass Helly den Durchtritt roter Blutkörperchen (auch Froschblutkörperchen) durch die unversehrte Wand der venösen Milzcapillaren gesehen hat und dass er hierin ein Hauptargument für die Lehre vom geschlossenen Kreislauf in der Milz erblickt.

To summarize, the injection method gives us extravasation. This has a positive value when physiologic or subnormal pressures are used. It proves the permeability of the vessel wall. For the structure of that wall, whether complete or incomplete anatomically, and especially for the ultimate morphologic fact, the form and relation of its constituent elements, it is valueless and these questions must be settled by direct observation, and not by indirect conjecture founded upon physical or physiological experiment. To quote Mollier again, referring to the question of open or closed circulation in the spleen:

Welcher Art diese Untersuchungen sein müssen, ergibt sich einmal aus dem Resultat der Injektionsversuche, welche lehren, das dieselben immer wieder sowohl zugunsten der einen wie der anderen Anschauung Beweismaterial geliefert haben. Sie sind also wohl nicht imstande, diese Frage zu entscheiden, und es ist besser, von ihnen zunächst ganz abzusehen.²⁰

His own brilliant solution of the problem of the structure of the sinus wall, is a ground of confidence to those who hold that structural problems are best approached by anatomic methods. In view of the complexity of structure and function in living beings, it will probably continue to be held the part of wisdom to determine their relation empirically and not attempt to deduce structure from the physical or even the physiological properties of organs and tissues.

The positive findings of the injection method, that there are in the embryo continuous vascular channels, that these channels have ends, and their walls are very pervious, can therefore be accepted for they are corroborated by other methods of investigation. The negative propositions that have been advanced by users of this method, that the embryo has only continuous endothelium, that its only source is from sprouts, that the ends of vessels do not communicate with tissue spaces, are not proved

²⁰ Loc. cit.

by the evidence which is alleged to support them. The method is partial in that it excludes a vasculogenetic periphery beyond its limit of exploration, uncritical in that it can only reveal permeability and not the structure of the vessel wall, and its advocates are inconsistent in that without just grounds they interpret its only peculiar datum, the extravasation, as an artifact.

Nor does the doctrine of specificity receive more support from observations *intra vitam*, if we distinguish between the growth and the formation of a tissue, and refrain from transferring processes recorded of a given stage, perhaps a late one, to earlier periods in ontogeny.

The observations upon the tails of amphibia, especially the tadpole, a classical object since the days of Schwann, have accumulated a rich fund of observations regarding the sprouting both of blood vessels and of lymphatics. Kölliker,²¹ Rouget²² and Golubew²³ followed the process, step by step, describing the hollow-pointed outgrowth, its acquisition of a lumen and its fusion with adjacent sprouts. The sprouts were found to arise, not only in the vicinity of nuclei but also at a distance from them (Köllicker, Flemming);²⁴ in the latter event nuclei were found subsequently to move into the sprouts (Fleming, Bobritski).²⁵ v. Ebner, in his edition of Kölliker's Handbuch, summarizes these findings and interprets them correctly as phenomena of growth:

Die Sprossung muss demgemäss als ein Wachsthumsvorgang des Protoplasma angesehen werden, denn auch in den Fällen, wo der Kern an der Sprossungsstelle liegt, lässt sich zunächst nichts von einer Kerntheilung sehen. Man muss wohl die primären Sprosse als sich aushöhlende Protoplasma knospen einer Endothelzelle ansehen. Erst sekundär erfolgt dann eine Mitose in der Zelle des Muttergefässes und der eine Tochterkern tritt nachträglich und nachdem er bereits wieder zu einem ruhenden Kerne geworden, in den Gefässspross, der unterdessen weiter gewachsen ist, ohne dass eine Zelltheilung erfolgt, da man, wie bereits erwähnt wurde, in jungen eben sich bildenden Kapillaren keine Zellgrenzen nachweisen kann.²⁶

²¹ 1846, Ann. S. sc. natur., T. v. p. 91. 1886, Zeitschr. f. wiss. Zool., Bd. 43, p. 1.

²² 1873, Arch. de Physiol.

²³ 1869, Arch. f. mikr. Anat., Bd. 5, p. 49.

²⁴ 1890, Arch. f. mikr. Anat., Bd. 35, p. 283.

²⁵ 1885, Central bl. f. mikr. Anat.

²⁶ Loc. cit., p. 672.

Schwann had believed that the capillaries were formed by the fusion of stellate cells, and Kölliker held at first the same view, but later abandoned it and considered the new formation of vessels in the tadpole's tail wholly due to sprouting.

Sigmund Mayer²⁷ made an important addition to our knowledge of the vascular system in his thorough study of its regressive processes. He distinguished between blood containing and bloodless vessels in the tadpole's tail, and pointed out that not all of the latter were to be taken as lymphatic, for many of them were degenerating blood vessels. The changes in the circulation incident to their collapse supports this view. His figures show many stages of their reduction and present striking resemblances to those of Fuchs, MacCallum and Bartels, before mentioned. Some confusion has been introduced into the terminology of these structures by Miss Sabin²⁸ who has invented the term "Mayer-Lewis anlage" to include these degenerating vessels and the endothelial sacs described by Lewis in the course of veins in the mammal. It will be remembered by the readers of F. T. Lewis'²⁹ valuable paper that he found the jugular lymph sac composed of several cavities, at first connected but later separated from the veins. Similar sacs were found adjacent to many of the veins, for which he assumed a like origin although he is most careful to state that in no single instance, other than at the jugulo-subclavian junction, was there any evidence of connection with veins.

Miss Sabin has further extended this concept to include the discrete mesenchymatous anlagen of the lymphatics, described by Huntington and McClure, which have at least a totally different structure from the formations described by Mayer. Their walls present every degree of transition from mesenchyme to fully formed endothelium and every degree of communication between their cavities and the spaces in the adjacent mesenchyme. They do not run off into long tapering processes, and they do not contain degenerating erythrocytes. It is highly probable

²⁷ 1885, Sitz-Ber. Akad. wiss. Wien. Bd. 91, p. 204.

²⁸ 1911, Anat. Rec., vol. 5, p. 417.

²⁹ 1906, Amer. Jour. Anat., vol. 5, p. 95.

that they are identical with the structures observed by Lewis, absolutely certain that they are not the same as the degenerating vessels of Mayer and Fuchs. The degenerating vessels described by Huntington in the cat have a wholly different appearance and to one familiar with the study of sections, the danger of confusing the two seems inconsiderable. With the aid of reconstruction and the comparison of closely graded embryos to ascertain the direction of the process, it is difficult to conceive of a possibility of so gross an error. In passing, I would note that the term *anlage* has hitherto been applied to the developmental process as indicating the earliest appearance of an organ. It is quite improperly used in connection with regressive structures.

More recently Clark³⁰ has published painstaking and elaborate studies of growing lymphatics in the tail of the tadpole, containing many interesting details and in major matters confirmatory of the work of his predecessors. We may therefore conclude that the vessels of the tail fin are formed by sprouting and that there is no evidence for the annexation of mesenchyme cells at this stage.

The debate is again not as to the fact, but solely in regard to the construction that shall be put upon it, and in particular the degree to which such observations may be extended to endothelium in general.

Goette³¹ described the first formation of vessels in amphibia from clefts in the mesoderm, which secondarily acquire a wall from adjacent cells and from intravascular wandering cells. Marshall³² and Morgan³³ have reached similar conclusions, and recently Studnička³⁴ has described the formation of the capillary as a channel in the mesostroma, into the wall of which cells migrate eventually forming an endothelium:

Ganz ähnlich verhält es sich auch mit den Blutkapillaren, welche jedenfalls sehr früh, wenn das Mesostromanetz noch ziemlich locker ist, zum Vorschein kommen. Die Bilder, die ich an meinen Objekten finde, sprechen durchaus nicht dafür, dass es ausschliesslich nur die Zellen

³⁰ 1909, *Anat. Rec.*, vol. 3, and 1912, *Am. Jour. Anat.*, vol. 13.

³¹ 1875, *Die Entwicklungsgeschichte der Unke*. Leipzig.

³² 1890, *Stud. Biolog. Lab. Owens College* 2: 1893. *Vertebrate embryology*.

³³ 1897, *The development of the frog's egg*. New York.

³⁴ 1911, *Anat. Anz.*, Bd. 40, p. 33.

wären, welche, wie man es meist annimmt, sich röhrenförmig zu Kapillaren umbilden würden; alles spricht dafür, dass die Kapillaren im Mesostromanetz auch dort, wo keine Zellen vorhanden sind, also ohne jede Beihilfe von Zellen weiterwachsen, und ihre Wand besteht da aus einer einfachen, zuerst plasmatischen Schicht, welche jener vollkommen entspricht, aus der sich z. B. das Corium bildet. Die Zellen sind in der Wand der jungen Kapillaren zuerst weit voneinander entfernt, und nur so, dass sie sich später vermehren und die innere Oberfläche der früher schon vorhandenen Kapillaren sekundär bedecken, kommt die Endothelauskleidung derselben zustande.

It is evident therefore that the processes in the tadpole's tail are not characteristic of all stages even in amphibia, and that much caution is requisite in attempting to generalize from such data. In the light of our present knowledge, far from extending these findings into a principle of vascular development, it would seem more prudent to seek to ascertain the peculiar conditions in larval organs which may serve to explain a deviation on the part of the mesenchyme cell from its usual behavior to developing vessels.

The transparent embryos of teleosts also afford a favorable opportunity for the study of living vessels. Wenkebach³⁵ has recorded important observations, especially upon *Belone longirostris*, and finds that mesenchyme cells are an important factor in the formation of vessels and sprouts.

Man sieht deutlich wie die Zellen, namentlich die des Mesoblasts, selbständig mittelst amoeboïder Bewegungen und oft ausserordentlich langer protoplasmatischer Fortsätze sich im Körper des Embryo und auf dem nicht mit Hypoblast umkleideten Dotter bewegen und nach bestimmten Stellen kriechen, als handelten sie mit Wille und Bewusstsein. Bei der Anlage und weiteren Ausbildung des Herzens, so wie bei der Bildung der Gefässe und anderer Organe spielt diese selbständige Bewegung der Mesoblastzellen eine grosse Rolle.

Nicht alle Zellen, die von dem Embryonalsaum auswandern bilden Pigment. Eine sehr grosse Zahl derselben zerstreut sich über die Oberfläche des Dotters und diese sind als Material für die später sich dort bildenden Blutgefässe zu betrachten.

Aus diesen Beobachtungen geht hervor, dass Mesoblastzellen durch selbständige amoeboïde Bewegungen die Wände der Blutgefässe des Dotters bilden.

Endlich werden die ersten circulirenden Zellen auch benutzt bei der Sprossenbildung der Dottergefässe. Die drei auf dem Dotter vorhan-

³⁵ 1886, Arch. mikr. Anat., Bd. 28, p. 225.

denen Gefässe fangen nämlich schon bald an kleine Seitenzweigchen zu bilden (Fig. 12 u. 13). Eine solche Sprossung wird eingeleitet von einer kleinen Ausbiegung der Gefässwand (verg. Fig. 25 a, b, c, d, e. und f.), an deren Spitze sich immer eine Zelle befindet. In dem Maasse als die Sprosse wächst, lagern sich immer neue, aber nicht sehr zahlreiche Zellen an dieselbe an, welche sich auf die schon oben beschriebene Weise ausbreiten, sich mit langen Fortsätzen an einander haften und so die Wand herstellen. Hierfür werden sowohl die vom Blutplasma herangeführten, als die sich schon auf dem Dotter befindenden Zellen verwendet. Diese letzteren Zellen scheinen eine grosse Neigung zu haben, Gefässe zu bilden, denn einige Male beobachtete ich, wie sich in diesem Stadium der Entwicklung freie, sich auf dem Dotter befindende Zellen, unabhängig von schon vorhandenen Gefässen, zusammenlagerten und kleine Röhrchen bildeten, welche später in das System der Dotterblutbahn eingereiht worden.

In diesem prachtvollen klaren Embryo sah ich auch die Bildung der kleineren Gefässe im Embryo selbst. Wie aus Figur 24 hervorgeht, bilden sich hier die kleinen um das Medullarrohr ziehenden Gefässchen ebenfalls aus Zellen, welche sich mittelst amoeboider Bewegungen zwischen den Theilen des Embryo hinziehen.

In their general features these observations were confirmed by Raffaele:³⁶

Le osservazioni fatte dal Wenckebach e già tante volte citate, e che io ho potuto in varie occasioni ripetere con risultati del tutto concordanti su varie uova di Teleostei, dimostrano chiaramente la migrazione di cellule mesenchimatiche in siti molto lontani dal punto di origine; ed è con questo processo che si formano tutti i vasi del vitello, compreso il cuore, il quale in taluni pesci ossei (*Belone*) si forma innanzi alla testa dell'embrione e in parte sul vitello.

From the foregoing, it would appear that in teleosts also there is the closest affinity between mesenchyme and endothelium, and that here sprouting from a formed and specific endothelium hardly plays a rôle in early stages of vascular formations.

A few other *intra vitam* observations may be noted briefly. McWhorter and Whipple were able to show and record photographically the concrescence of separate vascular anlagen in the area pellucida of the chick's blastoderm *in vitro*. This confirms Afanassiev's³⁷ and Klein's³⁸ observation of endothelial vesicles and seems an adequate refutation of Evan's opinion:

³⁶ Mitt. Zool. Stat. Neapol. Bd. 10, p. 441.

³⁷ 1869, Bul. de l'Acad. des Sc. d. St. Petersbourg, T. 13, p. 321.

³⁸ 1871, Sitz.-Ber. Akad. wiss. Wien, Bd. 63, p. 339.

Selbst auf dem Dottersack sind solche Gefäße, welche in loco entstanden sind, nicht zahlreich, denn sie entstehen nur an den Stellen der sogenannten "Anlagen," und die Hauptmasse des Kapillarplexus des Dottersacks entsteht durch Ausbreitung und reichliche Anastomosensbildung von den primären Gefäßen aus.³⁹

Further, the description by McWhorter and Whipple⁴⁰ of the to and fro movement of red corpuscles after the heart had begun to beat and prior to the establishment of the circulation points to their confinement in separate compartments. Their subsequent abrupt entry into the blood stream, must mean the giving way of the wall of the vesicles and their confluence to form vessels—all which is consonant with the data obtained from sections and reconstructions, and seems to afford a basis of interpretation for an interesting observation of Loeb's:⁴¹

It turned out in my experiments that the heterogeneous hybrids between bony fishes formed eyes, brains, ears, fins and pulsating hearts, blood and blood vessels, but could live only a limited time because no blood circulation was established at all—in spite of the fact that the heart beat for weeks—or that the circulation, if it was established at all, did not last long.

. I succeeded in producing the same type of faulty embryos in the pure breeds of a bony fish (*Fundulus heteroclitus*) by raising the eggs in 50 cc. of sea water to which was added 2 cc. one one-hundredth per cent, NaCN. The latter substance retards the velocity of oxidations and I obtained embryos which were in all details identical with the embryos produced by crossing the eggs of the same fish with the sperm of remote teleosts, e.g., *Otenolabrus* or *Menidia*. These embryos, which lived about a month, showed the peculiarity of possessing a beating heart and blood, but no circulation.

These findings of Loeb can hardly be reconciled with the doctrine that the vessels of the embryo have a primitive continuity of lumen with those of the yolk-sac, for it is inconceivable that in such circumstances, a beating heart could fail to effect a circulation. They are however corroborative of the observations of McWhorter and Whipple which would lead us to interpret the condition as a *Hemmungsbildung* due to failure of concrescence

³⁹ 1912, In Keibel u. Mall. *Entwicklungsgesch. d. Menschen*, Bd. 2, p. 552.

⁴⁰ 1912, *Anat. Rec.*, vol. 6, p. 121.

⁴¹ 1912, *Pop. Sci. Mo.*, vol. 80, p. 5.

between the separate anlagen of the vessels in the yolk-sac and in the embryo.

Very recently Miller and McWhorter⁴² have demonstrated the formation of the embryonic vessels *in situ* and their absolute genetic independence of those of the area vasculosa in the chick, by effecting a separation on one side between the body of the embryo and its membranes at a very early period, before vessels have appeared in the area pellucida. In their experiments, the blastoderm is incised longitudinally near the primitive streak. The wound gapes widely and the area vasculosa is separated from the embryonic body by a wide interval, which no sprouts cross. Notwithstanding which, the embryonic vessels are formed typically, and differ from those of the normal side only in size and rate of development, both of which details may be correlated to their reduced drainage area and the in consequence diminished quantity of circulatory fluid.

It would seem therefore that the limitations of injection and the data of *intra vitam* methods are hardly favorable enough to the doctrine of specificity, hardly discordant enough with the findings by the method of slides and reconstruction,⁴³ to attribute differences of opinion in these matters wholly to the methods

⁴² Abstract, Proc. Am. Ass. Anat., Anat. Rec., vol. 8, no. 2.

⁴³ E. R. Clark has endeavored (Anat. Rec., vol. 3, 1909) to show that fixation gives interruptions in the course of vessels which have previously been ascertained to be continuous by direct observation *intra vitam*. "A drawing was made of lymphatic and blood vessels during life. The tail was then cut into serial sections ten micra thick, stained in hematoxylin and Congo-red. In attempting to reconstruct it was found while blood capillaries, could often be fairly well constructed, it was impossible to reconstruct the lymphatics beyond the muscular margin." In a second paper (Anat. Rec., vol. 5, 1911) Clark shows evidence of retrograde progression in this line of technique, for the blood vessels in his 3, 6, and 7 figures can hardly be considered "fairly well reconstructed." There are no technical procedures that will not miscarry and such tests as Dr. Clark's are critical of personal accomplishment and judgment not of method in the abstract. In view of the technical difficulties offered by amphibian material, and the fact that none of the data on which the theory of condescence is based are derived from this form, Dr. Clark's criticism is both unfair and far from the point. With the same plausibility he might have proved that good sections could never be cut, because the yolk of frog's eggs is very likely to tear in paraffin. There is a relation between method and material, to disregard which for controversial purposes shows more of zeal than of sound reason.

employed. I have already expressed a doubt that this was the case and have suggested that different principles of interpretation were at the root of the difficulty, and I believe that the fault lies with the upholders of the doctrine of specificity, in their too great confidence in the uniformity and simplicity of ontogeny, in their failure to distinguish between genesis and growth, and in the prematurity of their assertion that endothelium is specific, before they have canvassed its potentialities and seriously studied its regressive phases.

The tendency to minimize the period of development, while the natural outcome of reliance upon injections which reveal only formed endothelium and continuity of lumina, has given the theory of specificity a strangely preformist character. Consistently carried out, it would reduce the history of endothelium to the expansion and ramification through the body of a single anlage, produced at a distance from the embryo in the mesoderm or from the entoderm of the yolk-sac. The innumerable separate anlages here, and their gradual concrescence are supported by a weight of evidence impossible to set aside, but the facts seem easier to be borne when this multiplicity is veiled as far as possible by using the term 'anlage' in the singular number and the collective sense. Yet even this 'anlage' proves unable to account for all the vessels of the embryo by its expansion, though Evans feels that most of those in the splanchnopleure are formed by sprouting. Exception must of course be made of the early ones in the area opaca, the 'anlage' itself, and those in the area pellucida which McWhorter and Whipple have shown are formed by concrescence.

Still there are further difficulties, certain vessels of the embryo arise *in situ*—the aorta in the head, the first arch, parts of the dorsal aorta, perhaps the heart; it is possible to attach too much importance to the early connection of the caudal portion of the aorta with the splanchnopleuric plexus (in chicks of 20 somites, Evans), and in the face of very general evidence of *in situ* origin it can only be maintained that any vessels which have not been individually proved to arise *in loco* are derived by ingrowth from the 'anlage,' with the lumen of which their lumina are shown by

injection to be continuous, when injection becomes practicable. From this moment (20 somites in the chick is the earliest recorded 'perfect' injection of Evans⁴⁴) there is a sudden and abrupt end to the production of discrete anlagen, contrary to what one would expect from the general gradual transitions in natural processes. All of the endothelium is now continuous, development in the sense of genesis has ceased, and growth alone takes place from this time forward. The moment at which injectibility is acquired is then the critical point in the whole history of endothelium.

But in view of the many separate anlagen, admittedly present in the splanchnopleure and in the embryo and of the transitions, almost universally observed in natural processes, the possibility must be conceded that a belated vesicle or two of endothelium might conceivably escape a sudden annexation to the injectible system. It becomes, therefore, a matter of interest to determine its status after injection has been practised and it has failed, hypothetically, to be injected. A moment before, had it been observed *in vitro*, or had the blastoderm been sectioned, it would have appeared like any of the other discrete anlagen in the 'anlage.' But the test of injection made, it at once ceases to have a future, it has become an artifact, or if pertinaciously insisted upon, a 'Mayer-Lewis anlage.' Because by a hair's breadth, by the veriest fraction of a second, it failed of concrescence prior to the moment of injection, it is absolutely devoid of vascular potentialities, and could never, had the injection been omitted, have joined its fellows and participated in the formation of the vascular system.

But our belated vesicle loses more than its future, it can hardly be said to have a real present existence. When the injection is made, it stands a chance of never being seen by the injector. The injection is then 'perfect.' If it is seen, it is an 'artifact,' or the injection is not 'complete.' If it is pointed out by another observer, then again the injection is incomplete, or it is a 'Mayer-Lewis anlage' and has lost a previous continuity with the injected vessels. All possibilities have been thoughtfully canvassed by the exponents of injection, and it can be confidently asserted that

⁴⁴ 1909, Amer. Jour. Anat., vol. 9, p. 281.

a perfect injection injects all the endothelium present; if more is found, then the injection is a failure but the principle remains unchanged.

There is, however, no reason to assume that injection has reached the acme of delicacy, great as is the skill which has been developed in its use. It is conceivable that some day it will be possible to inject a portion of the 'anlage' before its admittedly discrete vessels have fused—even a small fragment might be injected alone, the aorta in the head say, or the umbilical vein. This would then become to the injectionist the only source of the whole vascular system. The uninjected parts of the anlage would be demonstrably not vascular anlages at all, but artifacts or 'Mayer-Lewis anlages' and no study by slide or section could establish their right to serious consideration; they would be in the same status as the separate anlages of the thoracic duct, or any of the mesenchymatous vesicles of Huntington and McClure, and no degree of care in topographical localization, no minuteness in the gradation of reconstructed stages, no matter how fully their enlargement, gradual approximation and fusion were shown, would suffice to prove their participation in the formation of vessels. If not injectible they can never become endothelium. But the discrete vesicles and blood islands of the splanchnopleure are as an actual fact not injectible from the first and their structure is hardly known apart from sections. It would seem to be a reasonable and consistent conclusion from the injectionist standpoint, that the 'anlage,' in the splanchnopleure is an artifact or a 'Mayer-Lewis anlage,' and has absolutely nothing to do with the vascular system, which should consistently be held to come into being *ex abrupto* with the first moment of injectibility, for the chick, at present, the stage of 20 somites.

But some may not feel themselves obliged to confine their knowledge of endothelium to such facts only as can be corroborated by injection, for there is a stage of vascular genesis admittedly inaccessible to injection, and here the method of section and reconstruction has acknowledged validity and further is entirely consonant in its results with the data of *intra vitam* observation.

This granted, it is arbitrary to assert, that injection once it becomes practicable, dispenses us from the examination of the periphery beyond the terminus of the injection, where it is possible, where there is even antecedent probability that processes analagous to those in the 'anlage' continue and play an important rôle in the increase of vessels within the embryo. Whether this is true or not must be decided by the methods which do not prejudice the question. As in the case of the 'anlage' the results of the study of sections are in agreement with *intra vitam* findings, it is but reasonable in later stages and in the body of the embryo, where *intra vitam* methods are as yet inapplicable, to credit the method of sections and reconstruction with the same validity it is proved to possess in early stages.

To deny this, and insist that the limited field explored by injections is alone accessible to investigation, is not to assert the primacy of the injection method, but to fall back upon an ancient logical device known as the *petitio principii*.

The final objection and absolute disproof of the doctrine of specificity is the heterogeneity of the products to which endothelium gives rise.⁴⁵ I have already mentioned Huntington's description of the conversion to mesenchyme of regressive vessels in embryos of the cat (*vide ante*, p. 12).

Aurel von Szily⁴⁶ has described and illustrated the production of mesenchyme from the endothelial tube of the heart. And to pass over other investigators who have glanced the subject, Mall⁴⁷ has recently followed the process in detail and noted its theoretical importance.

The great importance of this distinction is at once apparent for it shows that connective tissue arises also from endothelial cells and that the intima of the entire vascular system including the valves of the heart has a like origin.

Further study will probably show that endothelial connective tissues are by no means of rare occurrence.

These observations of v. Szily, Huntington and Mall are of decisive importance and seem finally to dispose of the doctrine

⁴⁵ The idea that endothelium gives rise to connective tissue was clearly expressed as early as 1872 by Franz Boll. Arch. f. Mikro. Anat., Bd. 8, p. 53.

⁴⁶ 1903, Anat. Anz., Bd. 24, p. 417, cf. especially figure 2.

⁴⁷ 1912, Amer. Jour. Anat. vol. 13, p. 249.

of the specificity of endothelium, for it is fatuous to predicate specificity when products deviate in form and function from the type of the parent tissue.

Of even greater import, because evincing an identity of potentialities on the part of mesenchyme and endothelium, are the recent investigations in haematopoiesis, especially those of Maximow,⁴⁸ Dantschakoff,⁴⁹ Mollier⁵⁰ and Weidenreich.⁵¹ The last ('11) has published a comprehensive critical review of the modern literature of the white blood cells. The extent and variety of the evidence and the number of observers render impressive their concordant testimony to the mesodermal origin of blood cells, in the yolk-sac and the mesenchyme of the embryo, in the liver, spleen and lymphnodes, together with their frequent derivation from endothelium, and in a word the equivalence and permutability of mesenchyme, endothelium and blood cell. A few quotations from Weidenreich may serve to substantiate these statements (the italics are his):

Jedenfalls entstehen also schon in den *frühesten Entwicklungsperioden durch Abrundung und Loslösung aus dem Verband der Mesenchymzellen gleichfalls 'primitive Leucocyten,'* die teils den innerhalb der Blutbahn circulierenden völlig gleichen, teils in Grösse und Kernform differieren. Der Mutterboden dieser extravasculär gebildeten Elemente ist aber seiner Art nach durchaus derselbe, wie der der intravasculär entstehenden; denn sie entstammen beide mesenchymatösen Zellen, sei es nun, dass sie sich direkt aus den Blutinselementen absondern oder sekundär aus dem Endothel der Blutbahnen hervorgehen. . . . Es ist ferner von besonderer Wichtigkeit dass übereinstimmend angegeben wird, dass neben den *ungranulierten primitiven Leucocyten verhältnismässig frühe auch schon granulierten Formen* angetroffen werden, aber und das ist wesentlich *nicht innerhalb, sondern ausserhalb der Blutbahn;* es gehen

⁴⁸ 1906, Arch. f. mikr. Anat., Bd. 67, p. 608, 1907, Fol. haemat. Bd. 4, p. 611; and Beit. z. Path. Anat. u. allg. Pathol., Bd., 41, p. 122. 1909, Arch. f. mikr. Anat., Bd. 73, p. 444; and idem, Bd. 74, p. 525 and Fol. haematol. Bd. 8, p. 125; and Centralbl. f. allg. Pathol. u. path. Anat., Bd. 20, p. 145. 1910, Arch. f. mikr. Anat., Bd. 76, p. 1; and Verh. Anat. Ges. Brussel, S. 64.

⁴⁹ 1908, Anat. Hefte, Bd. 37, p. 473; Arch. f. mikr. Anat. Bd., 73, p. 117. Vehr. Anat. Ges. Ber., 1908, p. 72. 1909, Arch. f. mikr. Anat., Bd. 74, p. 855. Vehr. Anat. Ges. Brussel. 1910, p. 17.

⁵⁰ Loc. cit.

⁵¹ *Die Leucocyten und verwandte Zellformen.* Weisbaden 1911. *Sonderausgabe aus Merkel u. Bonnet, Ergeb. d. Anat.* Bd. 19, Abt. 2.

also hier die *granulierten Elemente* zunächst aus leucocytären *Wanderzellen* d. h. freigewordenen Mesenchymzellen hervor.⁶²

In the Liver:

Die '*primitive Blutzelle*,' die sich *abrundende Mesenchym-oder Reticulumzelle*, die *undifferenzierte 'Gefässwandzelle'* ist überall dasselbe Element, es erscheint bald als *Begrenzung einer Reticulummasche*, bald als *Wand einer schon etwas kanalisierten Blutbahn*, bald als *freie Blutzelle*; genau die gleichen Verhältnisse kehren also in der Leber wieder, wie man sie in den Blutinseln und Blutsträngen und den ersten Gefässanlagen des Mesoblasts findet, nur mit dem Unterschiede, dass hier Entodermzellen in Form der Leberanlage einwachsen und das Ganze mehr zerteilen. Als wesentlichstes Ergebnis ist sonach festzuhalten, dass *rote Blutkörperchen and Leucocyten von einer Zelle mit mesenchymatischem Charakter* gebildet werden. Die Frage, ob sie beide oder nur die eine extra, oder intravascular entstehen, ist nach der eben erörterten Auffassung ziemlich belanglos, da ja die Zelle, welche die Abgrenzung der primitiven Blutbahn nach innen und aussen bildet, eigentlich die Fähigkeit der Produktion beider Arten besitzt, eine Fähigkeit, die auch zunächst noch ihre freien Abkömmlinge bewahren.⁶³

In Bone Marrow:

Nach dieser auf genaue Detailbeschreibung und zahlreiche Abbildungen gestützten Auffassung entsteht also auch *im Knochenmark das blutbildende Gewebe durch Differenzierung aus losgelösten Mesenchymzellen vom Charakter kleiner und grosser ungranulierter basophiler Leucocyten*, die sich sowohl in der Richtung der roten Blutkörperchen, wie auch der weissen weiterdifferenzieren; noch ausgesprochener wie in der Leber erscheint dabei der ganze Prozess als ein *extravascularer Vorgang*.⁶⁴

In the Spleen:

So unbestimmt auch vorerst noch die Angaben über die Herkunft der Blutbildungszellen der Milz lauten, so scheint mir es doch im *höchsten Grade wahrscheinlich*, dass in der Milz wie in Leber und Knochenmark die Entwicklung ausgeht von *indifferenten basophilen grösseren und kleineren Zellen*, die als *abgerundete Mesenchymzellen oder Wanderzellen an Ort und Stelle sich bilden und sich dann zunächst sowohl in der Richtung der Erythrocyten wie der Leucocytenreihe (granulierte Leucocyten) differenzieren*. Dieses erste Elemente, die '*primitive Milzzelle*,' wäre also auch hier wieder die primitive Blutzelle bzw. der primitive Leucocyt oder *Molliers Hämogonie*. Die Umwandlung dieser Bildungszellen in die kleinen Lymphocytenformen im Gebiete der *Malpighischen Körperchen* setzt als *besondere Gewebsdifferenzierung* erst zu einen späteren Zeitpunkte ein; ich werde zeigen, warum das für die Gesamtaufassung der Lymphocytenfrage völlig gleichgültig ist.⁶⁵

In Lymphnodes:

⁶² Ibid, p. 188.

⁶³ Ibid, p. 196.

⁶⁴ Ibid, p. 199.

⁶⁵ Ibid, p. 201.

Aus allen diesen Angaben geht also hervor, dass die *freien Elemente der Lymphknoten an Ort und Stelle entstehen* und zwar wie überall aus abgerundeten und losgelösten Mesenchymzellen, die eine bestimmte Zeitlang auch hier die Fähigkeit haben, sich in der Richtung der roten und weissen Blutkörperchenreihe zu differenzieren. ⁵⁶

In Connective Tissue:

Als wesentliches Resultat aller dieser Untersuchungen ergibt sich also, dass dem embryonalen Bindegewebe zunächst im weitesten Umfange die Fähigkeit zukommt, aus seinen Mesenchymzellen freie bewegliche Blutzellen zu bilden, die den Typus der primitiven Blutelemente aufweisen und sich anfänglich sowohl in der Richtung der Erythrocyten wie der Leucocyten differenzieren können.⁵⁷

The doctrine of specificity has found its support in phenomena of growth, chiefly in the solid terminations of lymphatics which are observed in rather late periods of ontogeny, and the avoidance of these sprouts by mesenchyme cells in the tail of the tadpole. The former fact has been by implication projected into earlier stages of development, and into peripheral regions which are not accessible to the method of injection. The latter has been erected into a general principle of vascular development, in spite of facts of diametrically opposite import, both in the frog (Goette, Marshall, Morgan, Studnička) and in other transparent embryos (Wenkenbach, Raffaele).

The theory of adaptation rests upon the observation of the transformation of mesenchyme cells into endothelium in a large number of forms at various periods of ontogeny, ascertained by a variety of methods and by observers with very different problems in view; second on the reversion of endothelium to mesenchyme (v. Szily, Huntington, Mall): and third, on the community of products and the permutability of endothelium, blood cells and mesenchyme (Maximow, Dantschakoff, Mollier, Weidenreich et al.).

The doctrine of the angioblast is so closely entwined with that of the specificity of endothelium that there is little promise in an attempt to consider it separately, especially as this conception of vasculogenesis loses its chief *raison d'être* with the recognition of the plasticity (non-specificity) of endothelium. As presented

⁵⁶ Ibid, p. 203.

⁵⁷ Ibid, p. 205.

by Minot⁵⁸ the doctrine of the angioblast rests on two general propositions.

1. The early appearance of the angioblast and its possible entodermal origin:

Die vergleichende Embryologie lehrt uns, dass die ersten Gefässe gleichzeitig auf dem Dottersack entstehen. Sie bilden eine einheitliche Anlage, die wir dem Vorschlage von His folgend kurzweg Angioblast nennen. Es ist aber gleich hervorzuheben, dass mehrere Forscher, wie *Maximow* in seiner neuesten Schrift, Blutgefässe aus dem mesoderm des Embryo direkt entstehen lassen. In der Tat können wir die vollkommene frühzeitige Unabhängigkeit des Angioblastes von dem eigentlichen Mesoderm nur als höchst wahrscheinlich behaupten. Der Angioblast liegt ursprünglich unmittelbar auf dem Dotter und bildet ein Netzwerk, das fast so fort nach dem ersten Erscheinen der Anlage sich zu erkennen gibt. Das Mesoderm, sensu strictu, bildet eine zusammenhängende Schicht, die oberhalb der Gefässanlagen liegt, und nur in den Lücken des Gefässnetzes unmittelbar mit dem Dotter in Berührung kommt. Der Angioblast scheint, der grossen Mehrzahl der Angaben nach, bei allen Wirbeltieren sich direkt vom Dotter abzuspalten. Es ist also sehr schwer zu entscheiden, ob der Angioblast genetisch als dem mittleren Keimblatt gehörig oder als Abkömmling des Entoderms aufzufassen ist. Selbstverständlich gehen die Ansichten hierüber weit auseinander.

2. Its probable complete independence (specificity of endothelium).

Es bleibt aber die Tatsache, dass der Angioblast sehr frühzeitig selbstständig wird, und dass er das erste Gewebe des Embryo ist, welches eine unzweifelhafte Differenzierung und scharfe Abgrenzung aufweist.

Der Angioblast behält aller Wahrscheinlichkeit nach zeitlebens seine vollständige Unabhängigkeit. Mit anderen Worten, es ist wahrscheinlich, dass die Endothelien der Blutgefässe (und der Lymphgefässe) und die Blutzellen in jedem Stadium sämtlich direkte Abkömmlinge des primitiven Angioblastes sind. Leider aber erlauben uns unsere gegenwärtigen Kenntnisse nicht, mit absoluter Sicherheit eine Meinung hierüber auszusprechen.

It will be seen that the extremely problematic nature of both of these postulates is perceived by Minot and that his view of the derivation of blood and endothelium from the angioblast is hardly to be reconciled with the results of the careful and thorough investigations summarized by Weidenreich. One may

⁵⁸ Keibel a. Mall. Handbuch d. Entwicklungsgeschicht. d. Menschen, Bd. 2, p. 483.

share Dr. Minot's regret that the early closure ('09) of his chapter on the blood prevented him from availing himself of the fundamentally important data of recent haematology, but it is more seriously to be deplored that such far-reaching doctrines as those of the angioblast and of specificity should have been promulgated upon a narrow factual basis in a text-book that is sure to fall into the hands of the inexperienced when, at the time of publication, ('12), so much evidence was at hand which would seem to invalidate these hypotheses.

The statement (*vide supra*) that the testimony of the majority of findings supports a direct origin of the angioblast from the yolk must not be taken to imply an entodermal origin, for in this sense it is not substantiated by the literature as a few citations from the careful articles of Rückert and Mollier⁵⁹ may serve to show.

Amphibia, Urodeles:

Trotz vieler Bemühungen habe ich auch nicht zu entscheiden vermocht, ob an der Lieferung dieser Gefässzellen für das Dottergefässnetz (Fig. 692 gfg) ausser dem Mesoblast auch der Entoblast beteiligt ist. Ich glaube bestimmter nur sagen zu können, dass im Bereiche der epithelialen Splanchnopleura keine Gefässzellen vom Entoderm abgegeben werden, während viele Beobachtungen dafür sprechen, dass seitlich am Dotter dies vorkommt.⁶⁰

Anura:

Bei Anuren ist folglich die Blutzellenbildung vom histogenetischen Standpunkte entschieden eine vorwiegend entodermale gegenüber der vorwiegend mesodermalen bei Urodelen. Will man, um diesen Unterschied zu beseitigen, die Annahme von Schwink gelten lassen, dass es sich bei Anuren um eine verspätete Ablösung von eigentlich mesodermalen Zellen vom Entoderm handelt, so ist nichts dagegen einzuwenden und es mag dieselbe für manchen vielleicht eine gewisse Beruhigung sein.⁶¹

Cytostomata:

Durch diese lückenhafte Untersuchung einiger Entwicklungsstadien von Petromyzon sind wir doch zu dem Resultat gelangt, dass die Gefäss- und Blutbildung bei Cyclostomen in einfacherer Weise sich abspielt als bei Amphibien. Es kommt das vor allem darin zum Ausdruck, dass bei Cyclostomen die Aufgabe, Gefässe auf dem Dotter abzugrenzen, fast ausschliesslich noch der dünnen einschichtigen Mesoblastlage selbst zufällt, da nur stellenweise vereinzelte Gefässzellen auf dem Dotter sichtbar werden.

⁵⁹ 1906, Hertwig's Handb. d. vergl. u. exp. Entwickl. d. Wirbelth., Bd. 1, Th. 1.

⁶⁰ Mollier, loc. cit., p. 1068.

⁶¹ Mollier, loc. cit., p. 1074.

Die Entstehung der Blutzellen ist aber abhängig von der Art und Weise der Mesodermbildung, so dass der alte Streit, ob das Blut aus dem Mesoderm oder dem Entoderm sich bildet, gar nicht generell für die Wirbeltiere entschieden werden kann.⁶²

Dipnoi:

Es ähnelt also die Entwicklung mehr den Urodelen als den Anuren, da der Mesoblast frühzeitig vom Dotter sich trennt und die Gefässanlagen zunächst also ein mesodermales Produkt sind.⁶³

Selachians:

In welchem Umfang diese entodermale Gefässbildung stattfindet lässt sich nicht leicht abschätzen. Die Zahl der Sprossen an einer Keimscheibe ist eine mässige und an den verschiedenen Keimscheiben zudem wechselnde, aber andererseits zieht sich ihre Bildung über eine verhältnismässig lange Entwicklungszeit hinaus. Es ist auch diese Frage weniger von prinzipieller Bedeutung gegenüber der Konstatierung der Thatsache, dass überhaupt die mesodermalen Gefässanlagen von *Torpedo* sich durch entodermalen Nachschub ergänzen, eine Ansicht (Rückert 1887), die so allgemein bezweifelt worden ist, dass ich selbst mit einem gewissen Vorurteil gegen meine eigenen vor 18 Jahren angestellten Beobachtungen an eine erneunte Untersuchung des Gegenstandes herangetreten bin.⁶⁴

Man sieht hieraus, wie wenig Wert der viel diskutierten Frage ob der Meso- oder der Entoblast die Blutanlagen der Selachier liefert, zukommt. Es ist im Grunde nur Sache des Uebereinkommens welchen Namen man der betreffenden Zellschicht geben will. Entscheidet man sich zu Lehrzwecken für eines der beiden Blätter so wird man dem mittleren den vorzug geben dürfen, weil ein Teil der Inseln im selbständigen Mesoblast auftritt und die übrigen in einer Schicht die bald darauf zum Mesoblast wird.⁶⁵

Teleosts:

Als Produkt des peripheren Mesoderme wäre der intermediären Zellmasse zunächst die Fähigkeit zuzusprechen, Gefäss- und Blutzellen liefern zu können, und es wäre eine derartige Differenzierung derselben zu erwarten.⁶⁶

Es lässt sich zunächst also wohl auch die gefässbildende Kraft der intermediären Zellmasse als peripheres Mesoderm aufrecht erhalten, und es müssten diese Gefässe den Dottergefässen echter Meroblastier in der blutbildenden Zone verglichen werden.⁶⁷

Reptilia:

Was die genetischen Beziehungen der Blutinseln zu den Keimblättern anlangt, so leiten alle neueren Forscher diese Gefässanlagen aus dem Mesoblast ab, wie dies zuerst Strahl für *Lacerta* gethan hat. Das ist im Grunde auch die Meinung von Mehnert für die Schildkröte und von

⁶² Mollier, loc. cit., pp. 1088-9.

⁶³ Mollier, loc. cit., p. 1079.

⁶⁴ Rückert, loc. cit., p. 1111.

⁶⁵ Rückert, loc. cit., p. 1095.

⁶⁶ Mollier, loc. cit., p. 1145.

⁶⁷ Mollier, loc. cit., p. 1146.

Völzkow für das Krokodil, wenngleich beide Autoren den betreffenden Mesoblastabschnitt als Entoblast bezeichnet wissen wollen.⁶⁸

Birds:

Fassen wir das Gesagte zusammen, so hat sich ergeben, dass die soliden Anlagen der Dottergefäße des Huhnes ein verschiedenes Verhalten zum mittleren Keimblatt darbieten, je nachdem sie hinten oder vorn, aussen oder innen in der *A. vasculosa* auftreten. Diese Differenzen lassen sich aber alle leicht verständlich machen, wenn man von dem allerersten Entwicklungszustand derselben ausgeht und zugleich das jeweilige Verhalten des in ihrem Bereich befindlichen Mesoblast mitberücksichtigt; Die Gefässanlagen der *A. opaca* bilden sich, sie mögen hinten oder vorn auftreten, als Verdichtungen und Verdickungen eines vom Keimwall sich delaminierenden Primitivstreifenmesoblast. Sie scheiden dann an ihrer Oberfläche eine Mesoblastdecke ab, wodurch die unterbrochene Kontinuität des Blattes wiederhergestellt und sie selbst aus demselben ausgeschaltet werden. Die vorderen Anlagen unterscheiden sich von den hinteren genetisch darin, dass der Mesoblast sich von ihnen frühzeitiger differenziert und abtrennt, weil er vorn viel rascher der Cölombildung zustrebt. Die zu innerst in der *A. opaca* befindlichen Anlagen differieren von den übrigen, weiter peripher gelegenen, darin, dass sie infolge ihres späteren Auftretens in einem Mesoblast sich bilden, der seine Verbindung mit dem Keimwall bereits gelöst hat. Sie erscheinen daher als rein mesodermale Bildungen.⁶⁹

Mammals:

Nach dieser ausführlichen Behandlung der peripheren Mesoblastbildung können die Vorgänge, welche direkt zur Herstellung des Blutes und der Gefäße auf dem Dottersack der Säugetiere führen, kürzer dargestellt werden um so mehr, als dieselben bisher nur von wenig Forschern genauer untersucht worden sind. Hier ist wieder an erster Stelle Kölliker (1875 u. 1879) zu nennen, der beim Kaninchen zu dem gleichen Befund einer mesodermalen Blutbildung gelangt ist, wie beim Huhn. Seine Angaben wurden durch Heape (1887) für den Maulwurf, Robinson (1892) für die Maus, Janošik (1902) für Schwein und Ziesel, Fleischmann (1889) für die Katze und Keibel für das Meerschweinchen (1889) und den Menschen (1890) kurz bestätigt und vor allen in jüngster Zeit durch die ausführlichen Darstellungen Van der Stricht's für das Kaninchen (1895) und die Fledermaus (1899) erweitert.⁷⁰

Eine Beteiligung des Entoblast bei der Blutbildung durch Abgabe vom Zellen, schliesst Van der Stricht sowohl für das Kaninchen wie für die Fledermaus mit Entschiedenheit aus.⁷¹

An den genannten Orten gehen beim Schaf nach Bonnet die Gefäße aus vorgebildeten Räumen hervor, aus Lücken, die auf der Nabelblase zwischen dem Entoblast und dem visceralen Mesoblast, auf dem Amnion zwischen Entoblast und parietalem Mesoblast "ausgespart" und von den Mesoblastzellen allmählich "umscheidet" werden. Auf diese Weise entstehen blutleere, d. h. erythrocytenfreie Endothelröhren.⁷²

⁶⁸ Ruckert, loc. cit., p. 1193.

⁷¹ Ruckert, p. 1253.

⁶⁹ Ruckert, loc. cit., p. 1219.

⁷² Ruckert, p. 1254.

⁷⁰ Ruckert, loc. cit., p. 1251.

To return to Minot's conception of an angioblast, his description of its formation permits a somewhat more definite examination of his position.

Kurzgefasst findet die Differenzierung des Angioblastes bei Amnioten folgendermassen statt: Er bildet ein Netzwerk, das anfänglich aus Zellsträngen besteht, diese werden bald hohl; nach vielen Beobachtern kann die Höhlung auf der unteren Seite zuerst nur vom echten Dotter begrenzt sein. Die Angioblastzellen bilden sich zum Teil in Endothelzellen, zum Teil in junge Blutzellen (Mesamöboide) um. Das Endothel entsteht aus der peripherischen Lage der Stränge, die Blutelemente dagegen aus den mehr central gelegenen Zellen. Nur die Endothelien bilden ein ununterbrochenes Netz, die Blutzellen dagegen stellen zerstreute Haufen, die sogenannten Blutinseln dar.⁷³

In this passage a stratum is described interposed between the entoderm and the mesoderm (*sensu strictu*), which is evidently the Gefässblatt of many authors, a structure eminently vasculogenetic but not exclusively so as was accurately stated by Bonnet.⁷⁴ Its non-vascular products have generally been lost sight of, but its proendothelial stage is clearly recognized by Felix:⁷⁵

Die Darmarterien des Menschen entwickeln sich aus einem Gefässnetz, welches Darm- und Dottersack umspinnt (*rete periintestinale*). Fig. 539 zeigt die Rekonstruktion der Gefässe eines Embryo mit 5 bis 6 Ursegmentpaaren. Die Aorta ist bis zum zweiten Ursegment als Rohr mit allseitig geschlossener Wandung entwickelt, von da ab caudalwärts ist sie erst in der Anlage begriffen; sie erscheint hier auf dem querschnitt bald als geschlossener Ring, bald aus vereinzelter Zellen bestehend. Vom zukünftigen 7. Ursegment bis zur Schwanzspitze ist zwischen Dottersack bzw. Enddarm einerseits und visceralem Mesoblast andererseits ein Gefässnetz eingeschaltet, das *rete periintestinale* (Fig. 521 und 450). Im Bereiche des Mitteldarms sind die Maschen dieses Netzes schon mit deutlicher Wand versehen (dunkelrot in Fig. 539), im Bereiche des Enddarms haben wir noch gar keine Maschen, sondern nur ein einfaches Gefässblatt (Fig. 540), welches durch Delamination von dem visceralen Mesoblast abgelöst wird und noch keine einzelnen Gefässe entwickelt hat (blass rot in Fig. 539); in ihm verliert sich die aorta (Fig. 539). Aus diesem Gefässblatt entstehen also *rete periintestinale* und *aorta*.

⁷³ loc. cit., 484.

⁷⁴ 1889, Arch. f. Anat. u. Entwicklungsgesch. p. 1.

⁷⁵ 1912, Keibel and Mall Handb. d. Entwickschich. d. Mensch. Bd. 2, pp. 757-8.

More recently Bremer⁷⁶ has reconstructed what is essentially a prevascular stage of this layer.

I would emphasize the early stage and the cells which do not become endothelium, because I believe that in them we find an exit from the embarrassment of recognizing an independent angioblast which yet in its products is identical with mesenchyme, for we have too little evidence of convergence in ontogeny to make the idea of such an assimilation *a priori* attractive. It would seem, therefore, simpler to accept an origin of mesenchyme in the splanchno-pleure at an earlier period than has been universally recognized, by a migration of elements from the germ layers into the mesostroma as has been described by v. Szily,⁷⁷ Studnička⁷⁸ and others. The peculiarity of this district would then consist in its great degree of vascular productivity which might be occasioned physiologically by its relation to the yolk,⁷⁹ rather than determined genetically by a hypothetical derivation from the entoderm. We here approach the standpoint of Raffaele⁸⁰—“*le cellule endotheliali provengono dal mesenchime*”—and are encouraged to hope that eventually the formally genetic views embodied in the doctrine of the angioblast will give way to a more biological standpoint of interpretation, viz., that endothelium is the result of mechanical, blood or biochemical factors, both consequent on position in the broad sense of the term, and are neither of them rigidly predetermined to a special cytomorphosis by obscure causes associated with a peculiar provenience of their elements.

The mesenchyme has long been held to be a complex of variously derived elements, which owe their resemblance and solidarity to a common position, not to likeness of origin. That cells free themselves from germ-layers and migrate into the mesostroma between them is well established, and that vessels make an early appearance in these strata is indisputable, in the somato-pleure as well as in the splanchno-pleure. But that in the latter situ-

⁷⁶ 1912, Amer. Jour. Anat., vol. 13. p. 111. Vide addendum.

⁷⁷ Loc. cit., and Anat. Hefte, Bd. 33.

⁷⁸ Loc. cit.

⁷⁹ Cf. Ruckert, loc. cit., pp. 1180-81.

⁸⁰ Loc. cit., p. 446.

ation we have two independent subsidiary germ layers, mesenchyme and angioblast, which are absolutely distinct, though inseparably united, and are in no sense phases or modifications of one structural basis, is not an obvious conclusion and is insuperably difficult in the view of the later permutability of endothelium and mesenchymal derivatives.

The entodermal origin of some of these elements, the mesodermal of others, the great proportional variation of the two components in different ova, would seem to point to position in the broad sense of the term, and not to derivation as the determining factor in their behavior. The comprehensive and detailed resumé of Rückert and Mollier, together with their individual contributions seem to necessitate a view of this sort, for they definitively dispose of an entodermal or a mesodermal origin of vessels as a general principle of vasculogenesis.

* Clearly therefore, though the causative factors of vasculogenesis in the blastoderm are but imperfectly understood, yet in the light of present knowledge the source of the vasofactive cells must be conceived as of secondary rather than of primary importance. The doctrine of the angioblast can therefore find no support in the facts of comparative embryology in so far as it hints an entodermic origin for endothelium and blood. But if angioblast and mesenchyme are thus conterminous, arising from the same sources, and possessing like potentialities, and have further the most intimate topographical relations, it is difficult to see how they have come to be separated conceptually by some investigators, unless it be from their too intensive confinement of attention to the splanchnopleure, where the excess and precocity of vasculogenesis somewhat masks the presence of mesenchyme.

This is the phase of the problem which I have endeavored to approach directly in the light of observations upon a series of blastoderms of the domestic cat, ranging from the period of the three-layered blastoderm, to the stage of fourteen somites. They were fixed in Zenker's solution, embedded in paraffin, cut into serial transverse sections $13\frac{1}{2}$ μ thick, and variously stained—the most satisfactory results on the whole being gained with

Delafield's haematoxylin and eosin, or Heidenhain's haematoxylin. Reconstructions were made by the Born method in four cases; for others, in small easily defined areas, where conditions were simple, the quicker but less satisfactory graphic method was employed.

For convenience of analysis, the data are presented under two headings:

1. The early formation of vessels beneath the ectoderm, with special reference to the umbilical vein.
2. The early formation of vessels above the entoderm, especially the omphalo-mesenteric plexus and the aorta.

THE UMBILICAL VEIN

The early stages in the development of this vessel afford an opportunity to study the formation of endothelium in a circumscribed region and under comparatively simple conditions. It appears first near the lateral margin of the incipient intra-embryonic coelom and is separated from the entoderm by the whole thickness of the as yet imperfectly cleft mesoderm. The inner germ layer patently is excluded from direct participation in the formation of this endothelium, and its appearance, antedating the establishment of continuity with any derivative of the splanchno-pleuric plexus, must entail the acceptance of its origin *in situ*. I am not aware that the independence of the umbilical vein and the associated hypodermal vessels has ever been impugned. It was recognized by His and more recently has been considered by Mollier. In the cat it retains its isolation to the stage of 14 somites. It would seem, however, that its importance from the theoretical standpoint has not been universally appreciated. The position of the umbilical vein at the amnio-embryonic angle suggests that the vascular potentialities of intra- and extra-embryonic portions of the germ layers are not, of necessity, totally disparate. The extent of the vessel, its comprehensive drainage area, its high physiological importance, seem to require that its mode of formation be respected in general interpretations of vascular development. A doctrine such as that of the origin of endothelium from entoderm, or by the growth of anlagen formed in the splanchnopleure alone, neg-

lects the case of the umbilical vein, and is to that large degree incomplete and partial.

The youngest embryo which requires consideration here is No. 550 of the Columbia Collection. The somites are forming but as yet there is no complete intersomitic cleft; the coelom is little more than a line of beginning cleavage; in a few places the layers of mesoderm separate and narrow discontinuous clefts appear.

The parietal mesoderm is, in general, compact. It is separated from the ectoderm by a space which must be considered in large part due to shrinkage. In general, the outline of the mesoderm is clear cut, but lateral to the neural plate and dorsal to the coelom-clefts short protoplasmic processes extend from its peripheral cells into the hyp ectodermal space; a few are also to be seen on the basal surface of the ectoderm. These are evidently the fibers of Aurel v. Szily,⁸¹ the interdermal cytodesmata of Studnička,⁸² which collectively constitute the *mesostroma* of the latter investigator. Subsequently, these processes form a felt work between ectoderm and mesoderm, into which cells from the mesoderm migrate. The latter process, the formation of the somatopleuric mesenchyme, is in its inception in this embryo. We may distinguish first a loosening of the cells of the mesoderm characterized by an increased distance between the nuclei, the appearance of intercellular clefts, and the projection of an occasional nucleus beyond its fellows; second, the appearance of cells, or more strictly energids, connected by broad protoplasmic bridges with the mesoderm; in some cases the bridges are narrow, the energids are distinctly pedunculated, and finally there are a very few elements which are joined to the mesoderm by tenuous cytodesmata only.

In embryos in which the first intersomitic cleft is complete (Columbia Collection Nos. 449, 554, 555)•the mesenchyme has increased in amount and the area involved is larger, now extending mesad along the parietal mesoderm as far as the somites (fig. 1). The *mesostroma* is more abundant and in places forms a feltwork of protoplasmic strands. The dorsal contour of the

⁸¹ 1903, Anat. Anz., Bd., 24, p. 417.

⁸² 1911, Anat. Anz. Bd. 40, p. 33.

mesoderm is irregular: many of its elements project into the hyp ectodermal space, some broadly, some narrowly pedunculate, others connected to the underlying mesoderm only by fine cytodesmata. The cytoplasm of these energids is very scanty; the nuclei are large, vesicular, with one or sometimes a second, large spherical karyosome. In view of their scanty cytoplasm and the protoplasmic nature of the mesostroma there is no serious error in describing the process as a wandering of the nuclei, as Mall⁸³ does in the case of a similar phenomenon in the endocardium. In both cases mesenchyme is forming, here from mesoderm, there from endothelium; the latter therefore has not with its change of form lost the potentialities of mesoderm in this respect.

An embryo⁸⁴ (Columbia Collection No. 539), in which two complete intersomitic clefts are present shows a marked increase in the hyp ectodermal mesenchyme. Its distribution is shown in figures 2 to 4. In this case as in the following models, the compact mesoderm is reconstructed in white, in contradistinction to the mesenchyme which is green. The surface appears more uniform than is actually the case, for it was found impossible to retain the innumerable small strands of mesostroma and all the minute projections and irregularities that characterize the contour of the mesoderm in section (fig. 5). The model, therefore, shows only the topography of the mesenchyme. This is most abundant in the head region where the axial mesoderm is less compact than elsewhere, and along the lateral margin of the coelom in the plane of the somites and for some distance caudad. To a less degree, mesenchyme has formed from the parietal mesoderm and the dorsum of the somites.

In section, the somites have regular contours, but elsewhere the mesoderm gives rise to innumerable strands of mesostroma joining it to ectoderm. The mesenchyme elements have increased, and in addition to individually projecting cells, which show every degree of emergence from the mesoderm, larger processes of this layer extend into the hyp ectodermal space. These

⁸³ 1912, Amer. Jour. Anat., vol. 13, p. 249.

⁸⁴ I desire to express my appreciation and thanks to Prof. J. H. McGregor for the gift of this beautifully preserved embryo.

changes are at a maximum along the lateral margin of the coelom where the parietal as well as the visceral layers are fairly resolving themselves into mesenchyme, to an extent that threatens to interrupt their continuity—a conformation which characterizes this region until the coelom is fully formed. In individual sections there are many cells which appear free of the mesoderm save for cytodesmata. The study of serial sections shows that many of these are actually free (save for cytodesmata), but that others are the tips of the processes of mesoderm just mentioned. While the mesenchyme continues to be recruited by the migration of single cells, from this period on there occurs a more intensive process, in which projections are formed and separate *en masse*, and as these projections are inclined to the mesoderm at an acute angle and often extend through several sections, this phase of mesenchyme production may be described as delamination.

In the embryo of four somites (Columbia Collection No. 409) there is an interval between the mesenchyme of the lateral sheet, and that in the head and in the region of the nephrotomes, occasioned by the relative unproductiveness of the parietal layer of the cardiac coelom, which is thin and compact. The two areas become continuous opposite the last somite (fig. 6).

Our next embryo is of eight somites (Columbia Collection No. 530). The two areas of mesenchyme are now closer together and the parietal layer of the cardiac coelom is active in the production of this tissue (fig. 7).

In both of these embryos we find the same intimate relation between mesoderm and mesenchyme that has been described.

There are evidences of active migration of single energids into the mesostroma assisted by a coarser process of delamination. In addition, there are evidences of the inception of endothelium. Small clefts or cavities have appeared here and there amid the cells of the mesenchyme, which are thus coming to enclose minute vesicles. As these cells are in a syncytium, the question of the locality of the cleft, whether inter or intra-cellular is of secondary importance. The situation of these incipient vesicles (vaso-factive cells) is indicated by the yellow color in figure 7.

In figures 8 and 9 are shown sections of a vesicle in relation to the second nephrotome of the four somite embryo, which it will be seen has some superficial resemblance to a pronephric anlage. There is also a smaller one opposite the incipient fifth somite and a solid mass opposite the first somite. In the eight somite embryo, there is a similar sac-like anlage at the interval between the first and second somites; elsewhere along the nephrotomes are scattered mesenchyme cells. The formation of clefts has also been initiated in the lateral portion of the mesenchyme at several points.

The comparison between these embryos does not suffice to give a local individuality to the several endothelial anlages, it merely shows the inception of the latter tissue diffusely from mesenchyme. In older embryos the condition is found repeatedly and appears to be an intermediate stage between mesenchyme and endothelium, as it certainly is intermediate in form between the mesenchyme mass and the endothelial sacs; these cells of intermediate form are here designated vasofactive.

In the umbilical line, the formation of endothelium is accelerated cephalad; almost none is found caudal of the fifth somite in the ten somite embryo (Columbia Collection No. 532). Its distribution is shown in figure 10. There is abundant mesenchyme in the hypentodermal space as far caudad as the third somite. In its midst, but tending to a deep position near its junction with the compact mesoderm, are groups of cells with more abundant protoplasm which stains a little faintly. They form small masses, in intimate syncytial connection with the ordinary mesenchyme. In their interior are frequently small clefts. In some, the cavity is larger and the enclosing cells are flattened, eventually becoming thin and endothelioid (fig. 11). These last we may call angiocysts, using Bremer's term. The presence of several minute clefts or vacuoles in a single mass of vasofactive cells, suggests their formation by the running together of these small cavities. In figure 10 the ordinary mesenchyme is omitted, the clefts and cavities are shown in red, the protoplasm of their bounding cells in black. Opposite the fourth and fifth somites are scattered cells and cell-groups of a similar appearance in which cavities have not yet appeared. These are readily dis-

tinguished from the other mesenchyme cells by their transverse position, in addition to their slightly more abundant protoplasm and slightly paler stain. It is possible that the latter two characteristics may be due to the imbibition of fluid. The anlagen of the umbilical line are disposed along the lateral margin of the coelom. A mesal line of similar anlagen is forming above the nephrotomes, while a few scattered elements have an intermediate position. In all these situations the process is discontinuous, the masses of vasofactive cells as well as the cavities are separated by appreciable intervals in which ordinary mesenchyme is present. They can be made into a continuum only by the inclusion of this tissue. The result would be a sheet of mesenchyme studded with scattered endothelial anlagen as in the model of the twelve somite embryo (figs. 12, 13).

A second embryo of twelve somites (Columbia Collection No. 540) is shown in figure 14 for comparison with the embryo just described. The discontinuity of the anlagen is again apparent. In addition to those before mentioned in the umbilical line, in the cardinal line (along the nephrotomes) and intermediate between the two (somato-pleuric plexus), an angiocyst has formed just in front of the first somite. The transverse vasofactive cells have increased in number and are effecting junctions with one another and with the angiocysts. In a third embryo of 12 (Columbia Collection No. 536) somites, there were rather more numerous vasofactive cells, rather less developed endothelium but the general arrangement was very similar; the angiocyst in front of the first somite was also present.

The transverse position of the vasofactive cells requires explanation. They stand out clearly against the other mesenchyme cells which have their long axis in general radial, and are further distinguished by their rather deep position, near or in the almost cell-free interval between the bulk of the mesenchyme and the compact layer of parietal mesoderm. The nephrotomes are now resolving rapidly into mesenchyme, they are segmented, and are separating from the coelomic mesoderm; through the spaces thus formed, which contain only a loose mesostroma, the hypecto-dermal and hyperentodermal regions communicate. In the latter

the aorta, in the former the umbilical angiocysts are enlarging. The distending fluid must be derived from the adjacent region and its determination to these reservoirs must set up currents in the neighborhood. It is suggested that the conformation of the region (fig. 15) is favorable to the direction of such a current along the dorsal surface of the lateral mesoderm in the narrow interval between it and the bulk of the somatopleuric mesenchyme and that to this current the vasofactive cells owe their transverse orientation. Mesal to the coelom where the nephrotomes are resolving and where the current, if there be one, is directed ventro-mesad towards the aorta, the vasofactive cells have an oblique position. I have already referred to Bartels' view that the addition of cells to the tips of lymphatics is occasioned by currents; it would be satisfactory to think the same principles at work in both late and early stages of vascular formation and to find that the current, the *sine qua non* of the persistence of endothelium, is also its determining cause.

The vasofactive cells are dorso-ventrally flattened, they occur singly, in groups of two or three, continuous with, or wholly separate from the angiocysts. Where joined to the latter they could of course, in section be taken for sprouts, in spite of their flat plate-like form. But if more caudal regions of the same embryo are examined, or a corresponding region in a younger embryo, isolated flattened cells are found constantly, which I take to be evidence that the continuous plates, or if you will, cords are built up by the accretion of separate cells. When it is borne in mind that the distance from the umbilical to the cardinal line can be spanned by a half dozen of these cells, the opportunity of finding the angiocysts of these lines, continuous by means of strands of 'angioblast,' is not wanting. In one case (an embryo of 16 somites, Columbia Collection No. 551) I found such a cord extending from the umbilical vein to the aorta, which could have been taken to prove the origin of the aorta from the umbilical vein or *vice versa* by one who was apt at interpretation and had refrained from examining earlier stages.

In the 14 somite embryo (Columbia Collection No. 188) the umbilical vein has become organized in its whole length; there

are scattered but not very numerous anlagen in the cardinal line, and in the interval between the two. Many in the last position are now coalescing with the umbilical vein, which is assuming its function as a somatopleuric drainage channel. Caudally it turns dorsad in the wall of the amnio-embryonic cavity and terminates among vasofactive cell-masses. Conditions opposite the first somites are shown in figure 16. The dilated cephalic extremity of the umbilical vein shows evidence of its origin by coalescence in the presence of several perforations and septa. At the level of the third somite it has effected a connection with the omphalo-mesenteric vein which crosses it ventrad at right angles. The intervening compact mesoderm lateral to the coelom has given way over a small area (fig. 17) through which the anastomosis between the veins is established. Near the cephalic margin of the third somite a transverse vessel is forming and is continued forward obliquely to the front of the first somite where it is continuous with the channel along the neural tube. A comparison of these vessels with the angiocyts of figures 10 and 14 makes evident their correspondence in position. There is further a small communication between the transverse vessel and a branch of the aorta. Several small and as yet unannexed vesicles are present.

The whole length of the umbilical line in a twelve somite embryo (Columbia Collection No. 547) is shown in figures 12 and 13. The somatopleuric mesenchyme is seen from below, the mesoderm having been omitted as far mesad as the nephrotomes. Cephalad the vein is represented by a large multilocular cyst which extends from the limb bud, in which is a blood island, to the level of the fifth somite. Then follow a series of angiocyts, which are coalescing opposite the sixth and seventh somites but farther caudad are scattered and often widely separated. Vasofactive cells amid which clefts have appeared are shown in yellow. There are numerous discrete vesicles in the cardinal line, which being dorsal to the nephrotomes are not well seen in the figure. Opposite the last somites the mesenchyme forms a network of cords, the solidity of which is necessarily exaggerated by the reconstruction. The resemblance to Bremer's angioblast cords is striking.

Caudad the anlagen of the umbilical line are in the wall of the amnio-embryonic cavity (figure 18). The amnionic position of vascular rudiments has been described by Bonnet, Spee, and Keibel.⁵⁵ Although the position of the umbilical in the cat is really at the amnio-embryonic junction, and appears to be unusual only because of the moderate entypy of the blastoderm and the early closure of the amnion caudad, there are in addition endothelial sacs actually in the amnion itself as may be seen in figure 19 from a 16 somite embryo.

The foregoing data seem fully to warrant the conclusions that endothelium is formed directly from mesenchyme in the somatopleure and that vessels result from the concrescence of discrete anlagen. With these facts in mind we may approach the more difficult region of the splanchnopleure.

THE EARLY FORMATION OF VESSELS ABOVE THE ENTODERM

The formation of mesenchyme and endothelium in the splanchnopleure is essentially the same process as in the somatopleure; the differences are mainly in the proportions of the two tissues for here endothelium predominates and is associated with the extensive production of blood cells. In brief, there is a separation of cells from the mesoderm, by individual migration and extensively in groups and masses; these elements are interposed between the entoderm and the compact mesoderm, that is they come to occupy and finally obliterate the hyperentodermal space. The major part give rise to endothelium, and in the case of the larger masses (blood islands) to blood cells. Of the remainder, a considerable proportion subsequently become endothelium, but there is always a residuum of mesenchyme present. Later this becomes conspicuous and surrounds and embeds the vessels.

The process is initiated by the formation of blood islands. These appear as spindle shaped discrete thickenings of the mesoderm at a considerable distance lateral and caudal to the embryonic axis, in regions where the mesoderm is free and separated by an interval, probably much increased by fixation, from the entoderm.

⁵⁵ Quoted from Ruckert, loc. cit., pp. 1253-4.

The thickenings soon come to project from the ventral surface of the mesoderm, and begin to separate from it. At about the time when the first somites are forming and before definite inter-somitic clefts have appeared, the migration of energids becomes extensive and desquamation is also well marked. The uncleft mesoderm, the visceral layer of the incipient coelom, and the intermediate cell mass are all affected (fig. 20). Many strands of mesostroma cross the hyperentodermal space and among these the mesenchyme cells are scattered, singly or in flat plate-like groups. They show every degree of connection with the compact mesoderm, the plates in particular being often broadly connected. It is a repetition on a larger scale of the processes observed in the somatopleure, earlier in time, and more active. As yet no endothelium or blood cells have been formed.

In the model of the embryo of two somites (figs. 21 to 23) the distribution of the tissue in the hyperentodermal space is shown in relation to the compact mesoderm (white)—the mesenchyme is green, endothelium red, and the intermediate forms of tissue (vasofactive cells) yellow. Cephalad the line of the heart, ventral aorta and first arch is occupied by scattered mesenchyme cells and cell groups. There are also a few isolated mesenchyme elements of more mesal position. The line of the aorta is vacant to within a short distance of the first somite. Here scattered cells and plates are present. Ventral to the somites and immediately caudal to them an irregular continuous plate or strand has appeared. As yet this is connected with the more lateral mesenchyme in which the omphalomesenteric plexus is forming only at one point. There are, however, numerous patches of mesenchyme scattered in the interval. Caudad in the region of the primitive streak there is no axial mesenchyme. There is much mesenchyme, associated with vasofactive cells and endothelium, along the lateral margin of the coelom and extending mesad below the visceral mesoderm. This is most abundant caudad of the heart and the transverse plane of the somites, approximately in the region which Schwink⁸⁸ regarded as the point from which endothelium sprouts.

⁸⁸ 1891, *Morph. Jahr.*, Bd. 17, p. 288.

The embryo of four somites (fig. 24) shows a very considerable advance beyond this condition and illustrates the rapidity, almost abruptness, of the changes in vasculogenesis which have been described by Huntington.¹⁷ The heart which is now concealed in the myocardium is composed of a series of endothelial vesicles the walls of which are in contact and are often connected with processes of the mesoderm. In general structure it is not dissimilar to the segment of the omphalomesenteric vein with which it is continuous caudad. Cephalad the line of the ventral aorta is represented by plates of mesenchyme, vasofactive cells, and small endothelial sacs. A large multilocular cyst, showing evidences of concrescence in its septa, forms the cephalic element of the dorsal aorta. Laterad it is connected with the first arch which is already endothelial, and a similar connection is established with the juxta-neural line, i.e., the angiocysts are in contact, but their lumina are still divided by numerous delicate septa (fig. 25). A second endothelial sac in the aortic line is situated just cephalad of the somites, ending near the first somite in continuity with a flattened plate of mesenchyme cells.

Corresponding to the other somites are plates of mesenchyme, vasofactive cell masses with cleft-like lumina, and a few angiocysts. The omphalomesenteric plexus is composed of larger vesicles which are now coalescing, but their lumina are not as yet continuous with either the heart or the aorta. Between the mesal vasculogenetic line (the dorsal aorta) and the lateral, which comprises the ventral aorta, the heart and the omphalomesenteric vein and plexus, are scattered mesenchyme and vasofactive cells; cephalad these are more abundant and are now coalescing to bridge the interval between the dorsal and the ventral aorta. The first arch is endothelial and situated somewhat in front of the point at which connection is established with the juxta-neural line. Two other transverse strands are present, one largely mesenchyme, one of vasofactive cells.

¹⁷ Developmental relations between lymphatic and haemal vascular channels. Paper read at the XVII Internat. Med. Congress. London, August, 1913. To be published in the Am. Jour. Anat.

In the embryo of eight somites (fig. 26) a moderate advance has been made in organization and considerable changes in topography have been effected. The most striking at first glance is the increase in the internal limiting sulcus incident to the formation of the foregut. But it will also be observed that the heart has been carried forward and is now much nearer the blind extremity of the foregut than in the four somite embryo. The first somite is also at a position cephalad to that of the younger embryo. This might be taken to imply an addition of somites at the cranial end of the series, a most difficult point to establish in the absence of fixed landmarks and in the shifting topography of this region. But this assumption would not explain the advance of the heart. The increase of the mesoderm dorsad, and the formation of the foregut seem to call for a rearrangement of this layer and may possibly account for the shortening actually observed, though this naturally does not exclude the possibility of the production of somites from the head mesoderm.

The aortic angiocyts are confluent from the first arch to the fourth somite, and mesal to them are mesenchyme cells, many but not all of which are adherent to their walls. The heart is multilocular. The omphalomesenteric vein now has a continuous lumen and has effected several small connections with the omphalomesenteric plexus, which is now extensive. Amid its channels are scattered mesenchyme and vasofactive cells and numerous projections from the mesoderm, the tips of which on reaching the entoderm spread out, form horizontal processes, and partially enclose the endothelial channels (fig. 27). Subsequently these projections are resolved into mesenchyme in which the vessels are imbedded.

In the embryo of twelve somites (Columbia Collection No. 547) the dorsal aorta has acquired a continuous lumen from the first arch to the region of the primitive streak. Caudad it diminishes in diameter and terminates in connection with scattered angiocyts and vasofactive cells, which farther caudad give place to mesenchyme (fig. 13). The first arch is double, one arm passing laterad, one cephalad to the lateral cul de sac of the foregut. The ventral aorta is endothelial and at the sides of the gut is

connected with plates and strands of mesenchyme, which are the presumptive anlagen of the aortic arches and the adjacent mesenchyme. Amidst this tissue are a few blood islands.

The precise source of the cells which form the endothelium of the aorta is somewhat difficult to determine. The clear cut contour of the somites until the formation of the sclerotomes makes it difficult to believe that cells are here given off to the mesenchyme. Mollier has held that its material is derived from the intermediate cell masses, and has shown sections in which they are connected to the aorta by solid processes, similar to those which elsewhere give rise to mesenchyme and endothelium.

Such connections also are found in the cat. At the stage of eight somites, and more clearly in that of twelve (figs. 12 and 13) the aorta becomes joined to a more lateral point of the visceral mesoderm. From the convexity which this layer forms below the coelomic angle a ridge of cells projects ventro-mesad. This is separated in many places from the mesoderm *sensu strictu* by a narrow cleft, and thereupon comes into apposition with the aorta (figs. 29 to 33); in places the cells of this ridge become endothelial and contain a lumen which eventually communicates with that of the aorta. It is possible that the connection with the intermediate cell mass described by Mollier may be of a similar nature, and that in both cases we are dealing with an increment to already formed endothelium. The anlagen of the aorta both in front and behind the somites are freely connected with the overlying paraxial mesoderm and it is probable that they are directly derived from it prior to its segmentation. Later when the sclerotomes are formed the aorta enters into intimate relation to their loosely arranged cells (figs. 29 to 33). The addition of elements from this source to the aortic endothelium has been described by Mollier,⁸⁸ and his results are abundantly confirmed by conditions in the cat. Some of these cell masses acquire a lumen before addition to the aorta. In other sections the vessel loses its well defined outline mesad and its wall is completed by typical mesenchyme, with the interstices of which its lumen com-

⁸⁸ Loc. cit., p. 1267.

municates. Finally the mesal angle of the aorta is often occupied by masses of adherent blood cells, as though the sclerotome had given rise not only to endothelium but blood cells as well.

To summarize: the formation of endothelium in the splanchnopleure is precisely analogous to that in the somatopleure. Mesenchyme is formed *in situ* and secondarily in large part is transformed into endothelium. There are few solid strands of mesenchyme ('angioblast') and these are not primary but arise from the confluence of scattered plates of mesenchyme. They are rarely transverse, as though they had grown in, but conform to the lines of future vessels. More important than their frequent interruptions as evidence of *in situ* origin, are their innumerable connections with the compact mesoderm, every degree of emergence from which, both by migration of single energids and by delamination, is conspicuous in every embryo examined.

ADDENDUM

Since the foregoing paper has been in proof a communication made by Bremer at the thirtieth session of the A.A.A. in Philadelphia and published in abstract (Proc. Am. Ass. Anat., Anat. Rec., vol. 8, no. 2) makes a brief consideration of the results of this investigator desirable. In his first paper (Am. Jour. Anat., vol. 13, 1912) Bremer describes the development of the intraembryonic blood vessels in the rabbit as resulting from the ingrowth of a continuous network of "angioblast cords" derived from the extraembryonic plexus of the yolk-sac. Although dealing in this paper with the development as found in rabbit embryos he examined other species, as chick, pig, sheep, etc., and felt "satisfied that in all essential points the story of the development of these primary vessels in other vertebrates will be found similar. . . ." The evidence on which these conclusions rest is briefly the continuity of the aortic and cardiac angiocyts with the extraembryonic vessels of the splanchnopleure by solid cords of cells, which are interpreted as angioblast. The essential data are given succinctly and the principle of his interpretation made clear in the description and figure of the rabbit embryo of 5 segments. "Fig. 1. a reconstruction of the angioblast cords of one side of a rabbit embryo of five segments, shows these cords, streaming in from the network over the yolk-sac. . . . They have grown from left to right of the figure, occasionally anastomosing until near the median line, which lies at the left of the figure. The shape of the meshes of this net indicates it seems to me the direction of this growth and the rapidity with which it has occurred." I question both the angioblastic nature

of these cords and the principle on which they are interpreted as ingrowths. Continuity of structure at a given period of development can not be held to establish the presence of continuity at earlier stages, for it may be secondarily achieved, as is shown by the union of the mesonephric tubules with the pronephric duct, to instance but one of several similar junctions in the development of the genito-urinary tract. In this particular case I must consider both the assumption of primitive continuity and of ingrowth wholly invalidated by the experiments of Hahn (*Arch. f. Entw. Mech. I Organismen Bd. 27, 1909*) and the recent very satisfactory confirmatory results of Miller and McWhorter, which after some amusing editorial adventures in the office of the *American Journal of Anatomy* were presented at the thirtieth session of the A.A.A. (*Proc. Am. Ass. Anat., Anat. Rec., vol. 8, no. 2, p. 91*). As to the nature of the cellular cords designated angioblast, it is to be noted that Bremer does not figure or describe either mesostroma or mesenchyme in his embryos. As he does not distinguish these formations, it is possible that they are included in his angioblast cords, which would then represent an early for the most part pre-vascular stage of the splanchnopleuric mesenchyme. I am inclined further to this view by the loss of cords in the embryo of 6 to 7 segments as evidenced in the comparison of figures 1 and 3, and to which Bremer calls attention in his text. The most plausible explanation would be that they had resolved themselves into mesenchyme; that therefore the primitive network was mesenchyme, which in widely separated regions was partially transforming itself into endothelium.

I am interested to note that in his second paper, a highly interesting study of early vessels in man (*Proc. Am. Ass. Anat. 30th Sess. Anat. Rec., vol. 8, no. 2, p. 97*) Bremer abandons his original position and accepts the development of discontinuous vascular anlagen independent of the vessels of the yolk-sac, but nevertheless retains the principle of growth in continuity in the vascularization of the embryonic body. With the general principles of blood vessel origin from mesoderm I am in hearty agreement, as also with the multiplicity of anlagen, and their intimate relation to the coelomic mesoderm. In these points there now seems to be a very general agreement between the students of mammalian vasculogenesis. But I must question the validity of a continuous angioblast network "composed of angiocysts and solid cords," which appears superfluous in view of Bremer's conclusion that vessels "originate from ingrowths of mesothelium as a number of separated cords, angiocysts, or blood islands, which are soon connected by sprouts of endothelium." In the interpretation of the solid strands connecting angiocysts as 'angioblast' Bremer states that he relies on his earlier work upon the rabbit, and I would again express my dissent. The mesenchyme and mesostroma form a protoplasmic continuum in which are contained angiocysts. To designate such portions of it as intervene between two angiocysts "angioblast" is in my opinion to make a distinction which does not exist, and which would not suggest itself in regions where the mesenchyme preponderates over the blood vessels as in the somatopleure.

• PLATES AND FIGURES

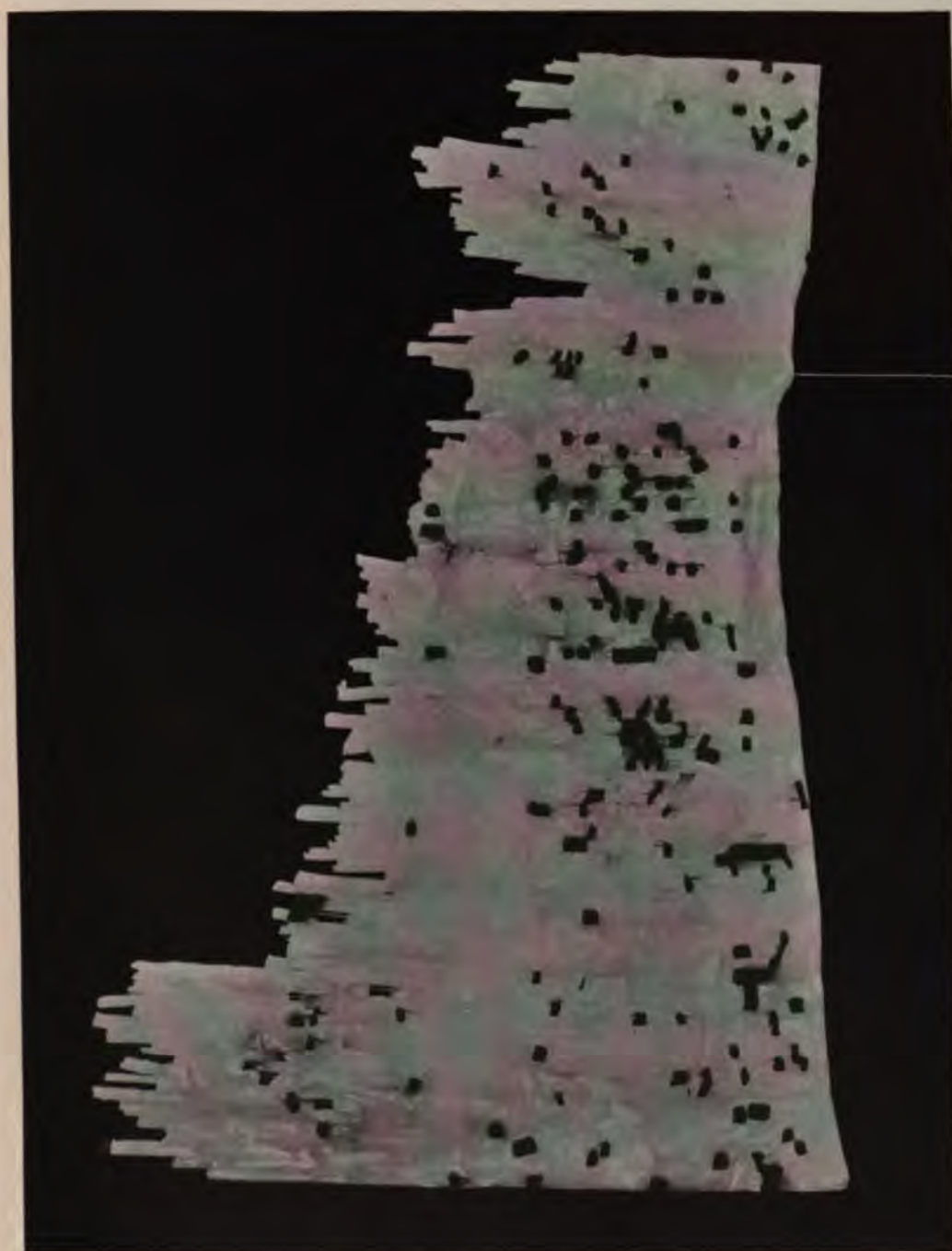


Fig. 1 Transverse section of an embryo with one pair of intersomitic clefts. Columbia collection No. 554, slide 4, row 5, section 8, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|----------------------------|------------------------------|
| 1 Ectoderm | 5 Hyperentodermal mesenchyme |
| 2 Mesoderm | 6 Mesostroma |
| 3 Entoderm | 7 Coelom |
| 4 Hypertodermal mesenchyme | 8 Neural plate |

Figs. 2 to 4 Dorsal view of a model of the mesoderm and mesenchyme of an embryo of two somites. Columbia collection No. 539, $\times 300$. Reduced $\frac{1}{2}$. The mesoderm *sensu stricto* is white, the mesenchyme green.

- | | |
|---|--|
| 1 First intersomitic cleft | 4 From this point caudad the mesoderm is continuous across the median line |
| 2 Second intersomitic cleft | |
| 3 From this point craniad the mesoderm is continuous across the median line | |



3

Fig. 3 Dorsal view of a model of the mesoderm and mesenchyme of an embryo of two somites. *1*, First intersomitic cleft; *2*, second intersomitic cleft.

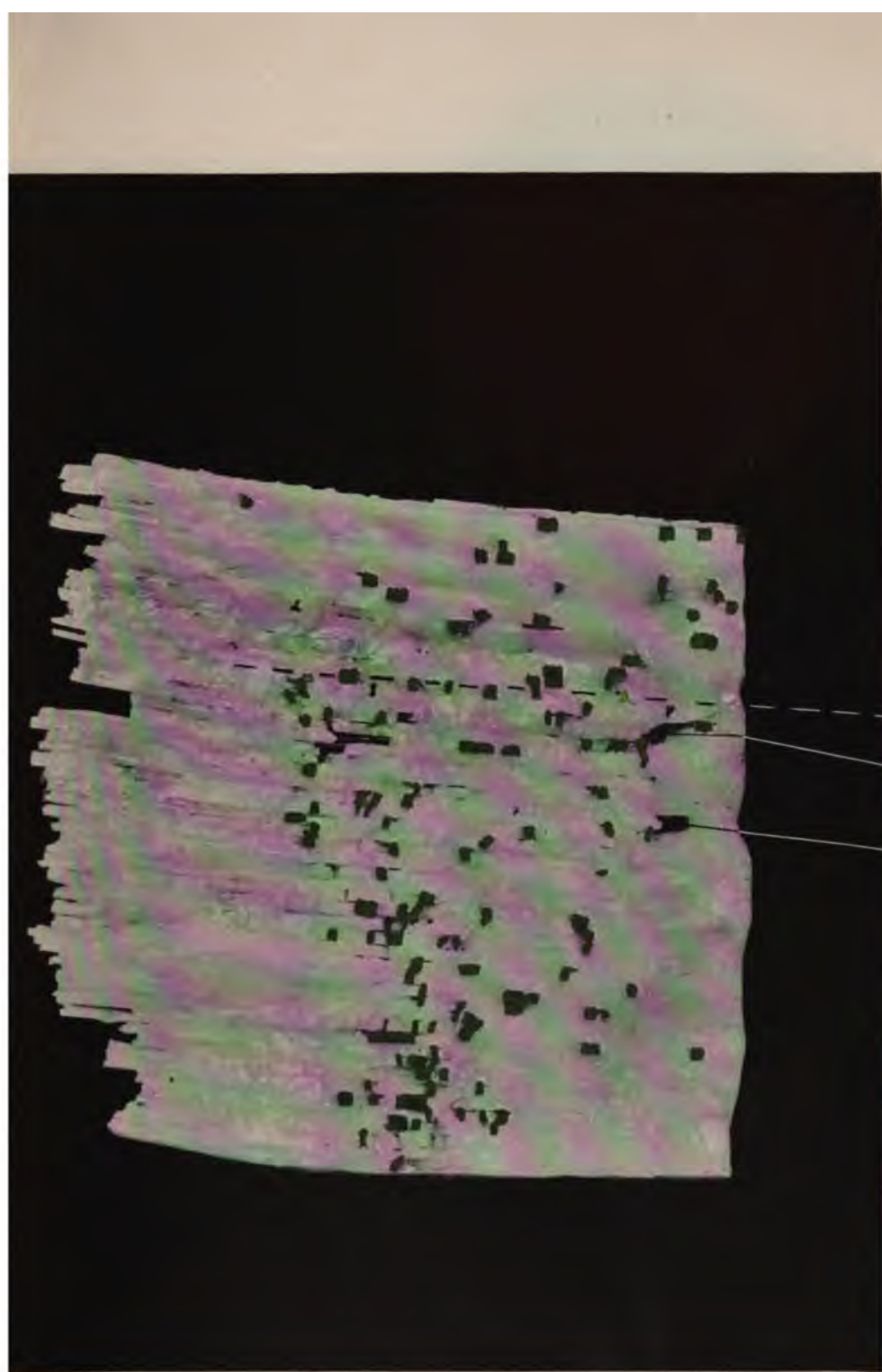


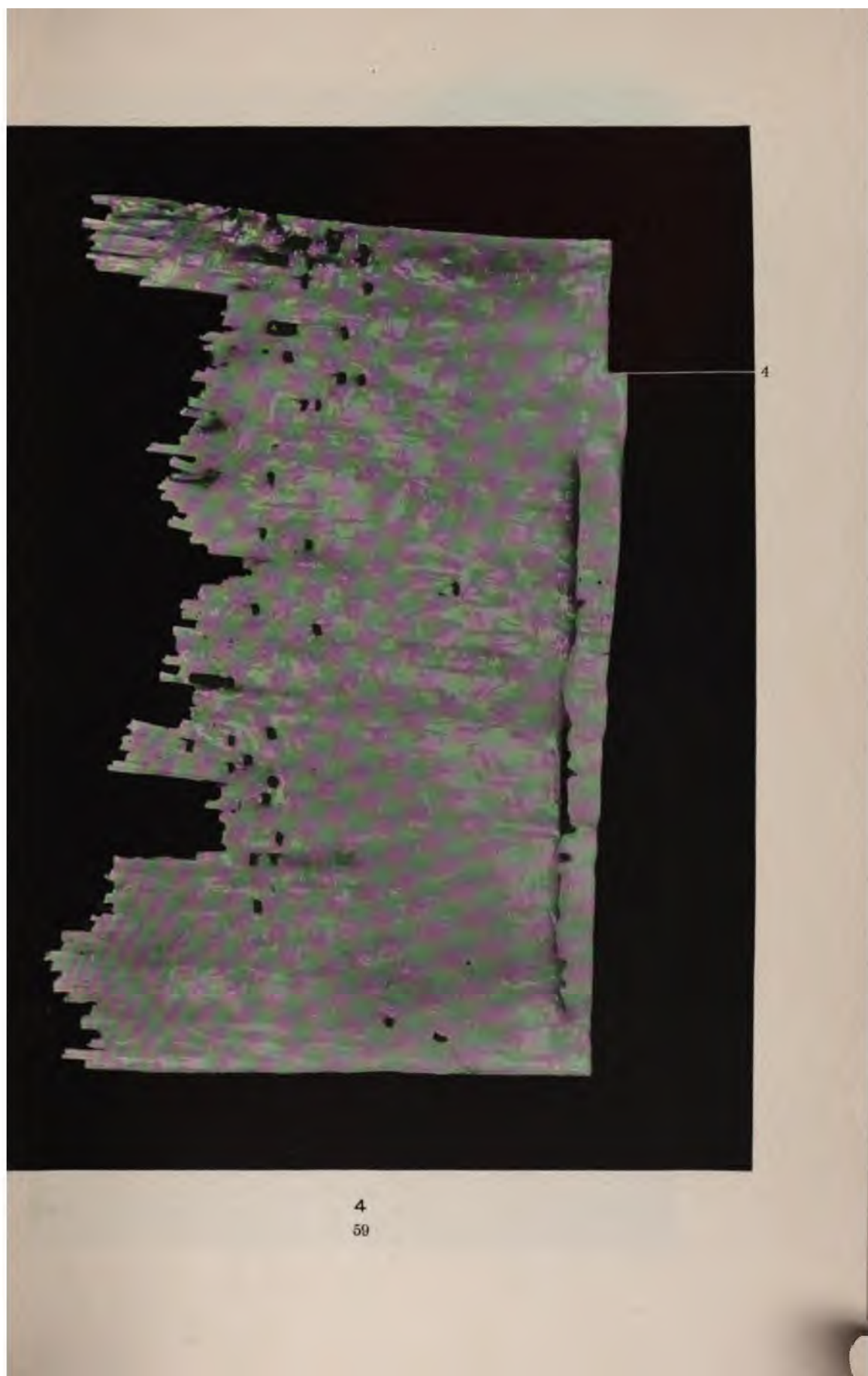
FIG.5

1

2



Fig. 4 Dorsal view of a model of the mesoderm and mesenchyme of an embryo of two somites. 4, From this point caudad the mesoderm is continuous across the median line.



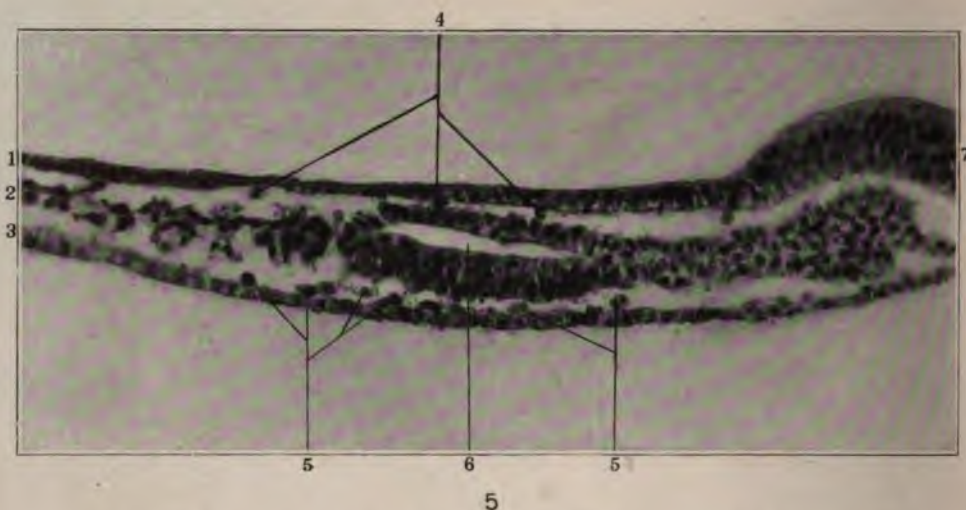


Fig. 5 Transverse section of an embryo of two somites at the level indicated in Figure 3. Slide 5, row 1, section 6, $\times 300$. Reduced $\frac{1}{2}$.

- | | |
|----------------------------|------------------------------|
| 1 Ectoderm | 5 Hyperentodermal mesenchyme |
| 2 Mesoderm | 6 Coelom |
| 3 Entoderm | 7 Neural plate |
| 4 Hypentodermal mesenchyme | |

Fig. 6 Dorsal view of model of mesoderm (white), mesenchyme (green) and vasofactive cells (yellow) of an embryo of four somites. Columbia collection No. 409, $\times 300$. Reduced $\frac{1}{2}$.

- | | |
|--|--|
| 1 Somite 1 | 4 Site of quintal ganglion |
| 2 Anlages of juxta-neural anastomosis | 5 Endothelial anlage opposite the second nephrotome; cf. figures 8 and 9 |
| 3 Connection between the juxta-neural anastomosis and the aorta, cf. figure 25 | 6 Region of cardiac coelom |

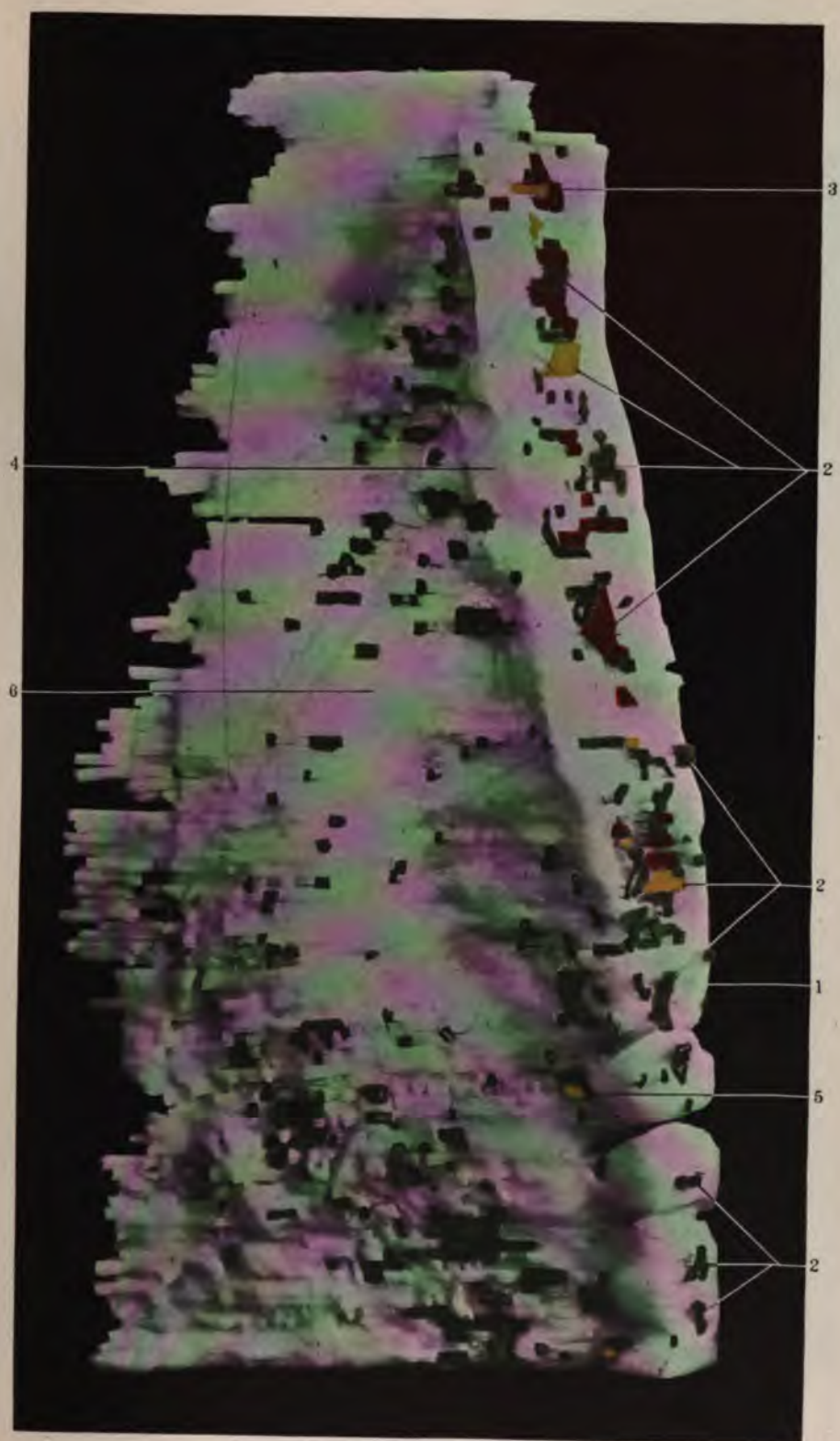
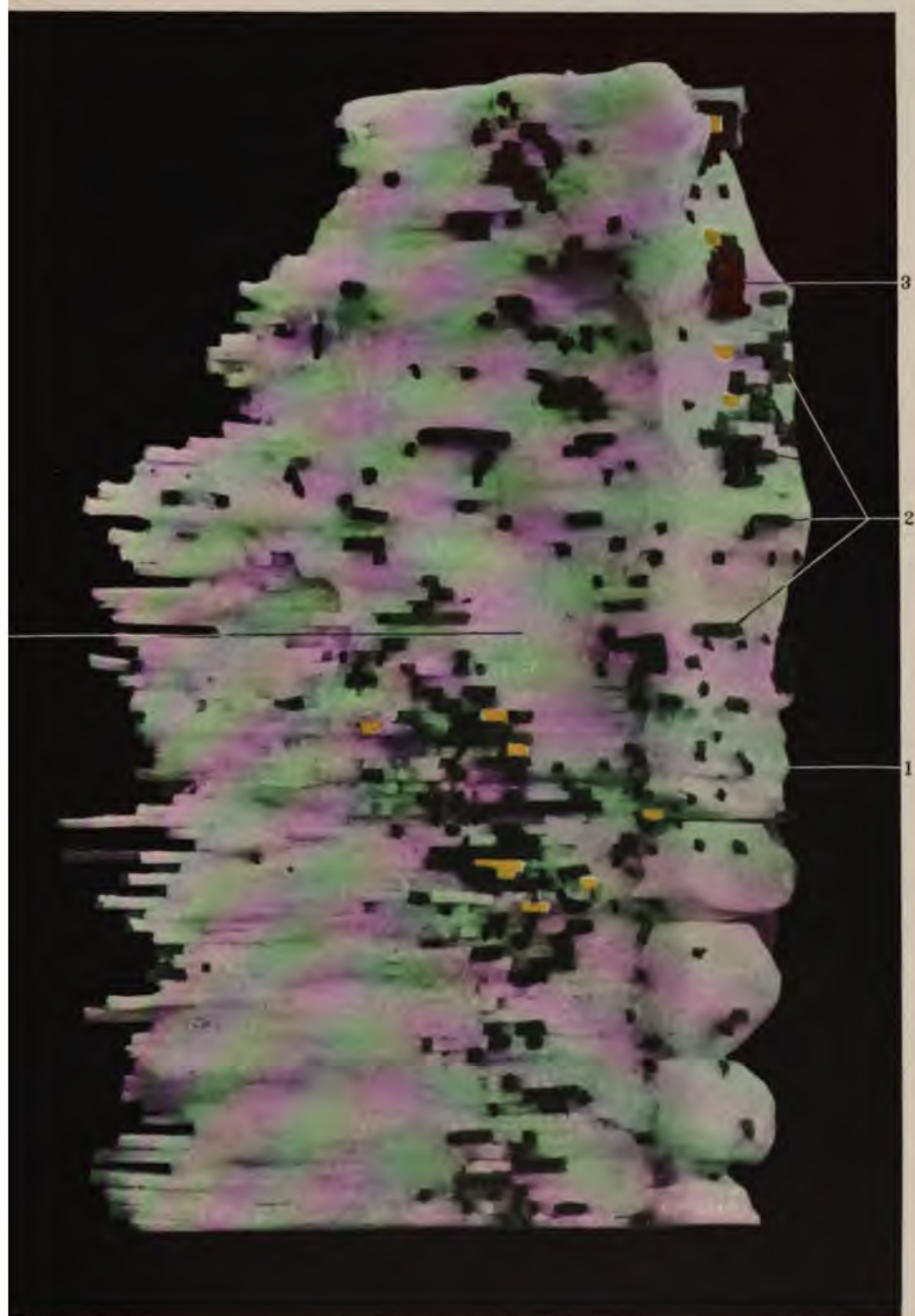


Fig. 7 Dorsal view of model of mesoderm (white), mesenchyme (green), vasofactive cells (yellow) and endothelium (red) of an embryo of eight somites. Columbia collection No. 530, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|---|----------------------------|
| 1 Somite 1 | 4 Region of cardiac coelom |
| 2 Anlages of juxta-neural anastomosis | |
| 3 Connection of juxta-neural anastomosis with the aorta | |





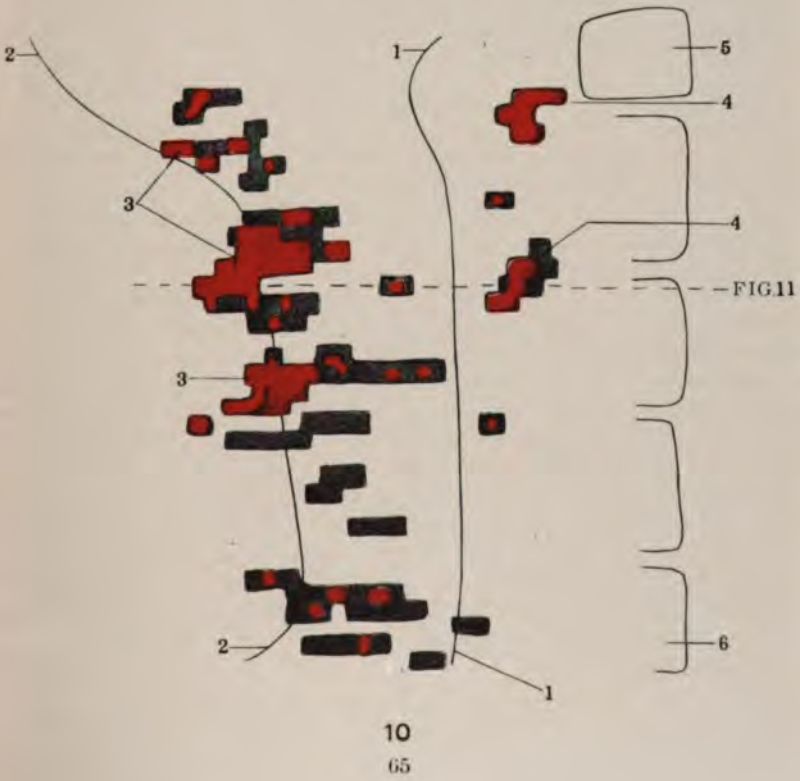
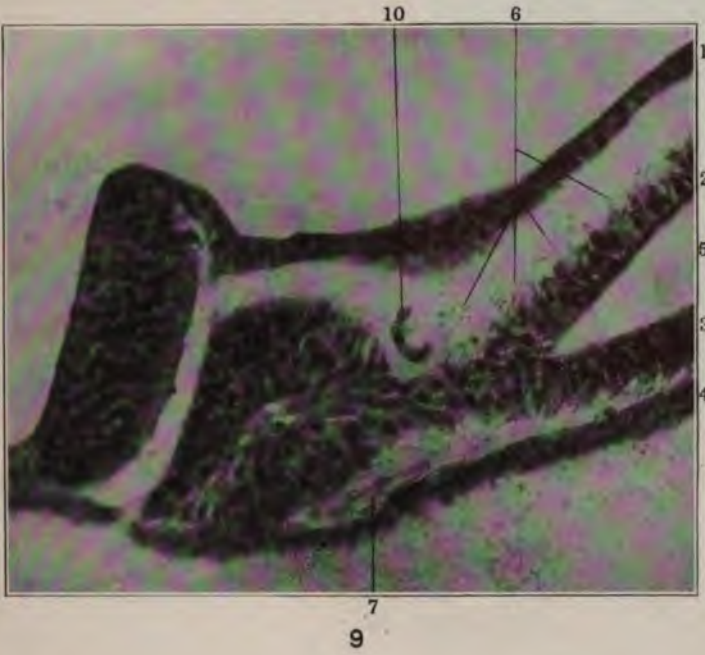
8

Figs. 8 and 9 Successive transverse sections of Embryo No. 400 passing through the second somite. Left side. Slide 4 row 6, section 12 and row 5, section 1, $\times 300$. Reduced $\frac{1}{4}$. To show derivation of endothelium from intermediate cell mass.

- | | |
|---------------------|--|
| 1 Ectoderm | 8 Intermediate cell mass |
| 2 Parietal mesoderm | 9 Projection from intermediate cell mass |
| 3 Visceral mesoderm | 10 Endothelial vesicle continuous with the projection from intermediate cell mass of the preceding section |
| 4 Entoderm | |
| 5 Coelom | |
| 6 Mesostroma | |
| 7 Dorsal aorta | |

Fig. 10 Graphic reconstruction showing the distribution of endothelium and vasofactive cells in the somatopleure of an embryo of ten somites. The cavities of angiocyts and clefts in vasofactive cells are shown in red. Columbia collection No. 532. Left side, dorsal view, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|-------------------------------|------------------------------|
| 1 Mesal margin of coelom | 4 Angiocyts of cardinal line |
| 2 Lateral margin of coelom | 5 Somite 1 |
| 3 Angiocyts of umbilical line | 6 Somite 5 |





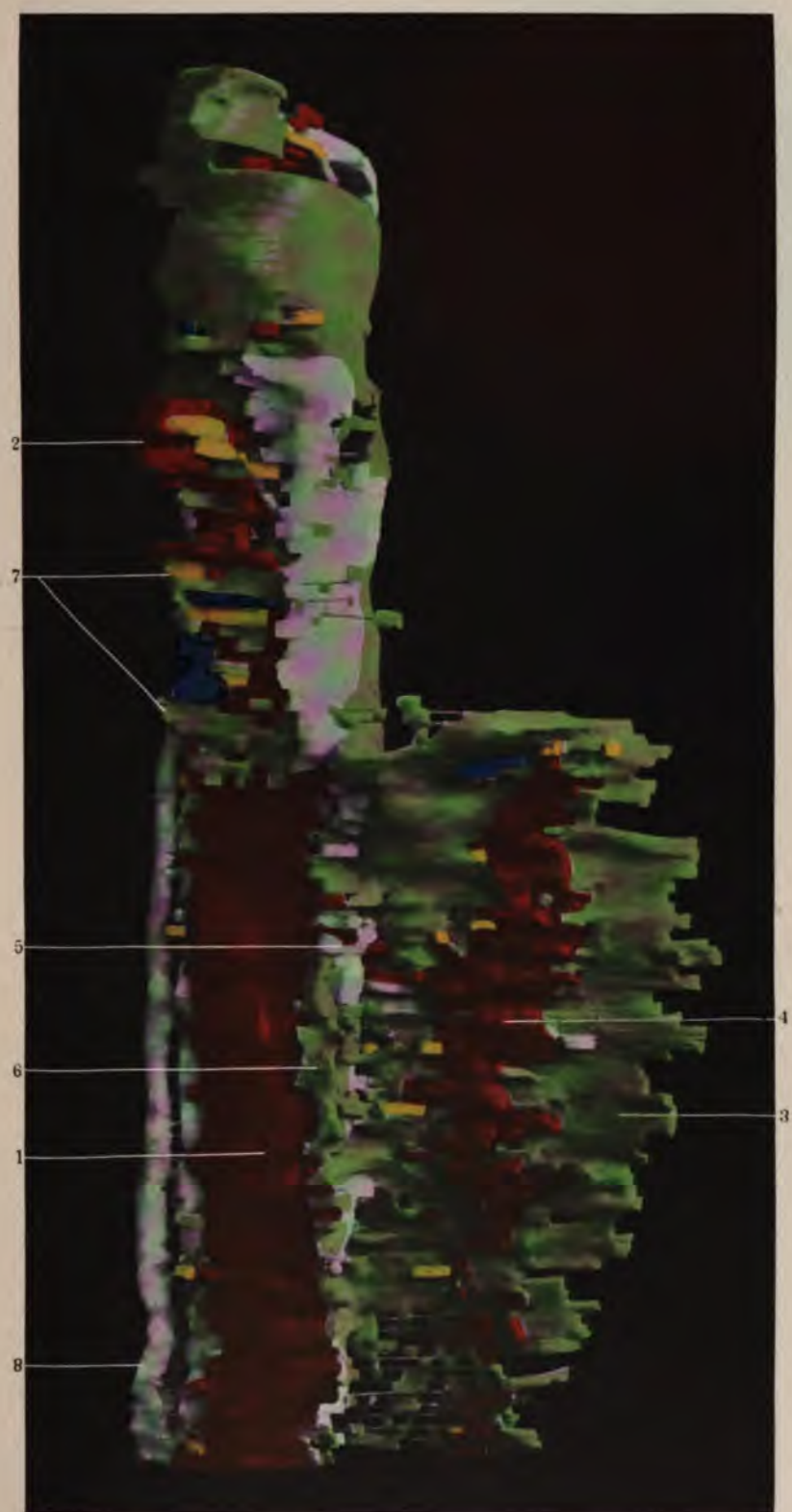
11

Fig. 11 Transverse section of same embryo at level indicated in preceding figure, $\times 300$. Reduced $\frac{1}{4}$. Slide 6, row 2, section 8.

- | | |
|-------------------------------|--------------------------------------|
| 1 Aorta | 4 Angiocyst of cardinal line |
| 2 Omphalomesenteric vein | 5 Angiocyst of intermediate position |
| 3 Angiocyst of umbilical line | 6 Coelom |

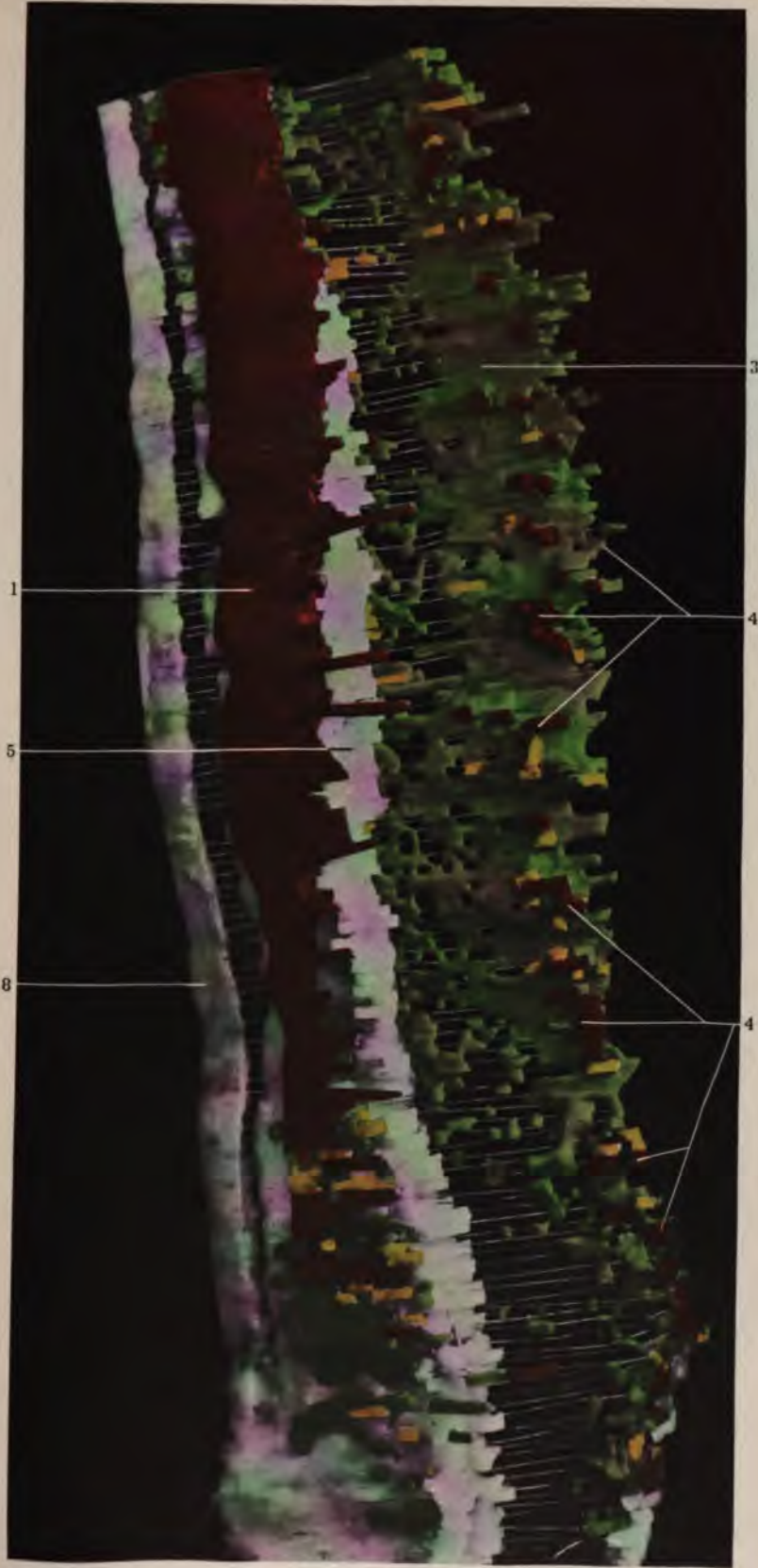
Figs. 12 and 13 Ventral view of model of mesoderm, mesenchyme and vascular anlagen of an embryo of twelve somites. Columbia Collection No. 547. The coelomic mesoderm from the nephrotomes laterad has been omitted, to allow the hyp ectodermal mesenchyme (green) to be seen from below, with its endothelium (red), vasofactive cells (yellow) and blood islands (blue), $\times 300$. Reduced $\frac{1}{2}$. The umbilical vein extends into the incipient limb bud.

- | | |
|--------------------------------|--|
| 1 Dorsal aorta | 6 Cells derived from visceral mesoderm in process of accretion to the aorta cf. figure 28 |
| 2 Truncus arteriosus | 7 Mesenchyme about foregut containing anlagen of ventral aortic root and caudal aortic arches. |
| 3 Hyp ectodermal mesenchyme | |
| 4 Angiocysts of umbilical vein | |
| 5 Coelomic angle | |



12
67

Fig. 13 Ventral view of model of mesoderm, mesenchyme and vascular anlagen of an embryo of twelve somites. *1*, Dorsal aorta; *3*, hypodermal mesenchyme; *4*, angiocytes of umbilical vein; *5*, coelomic angle.



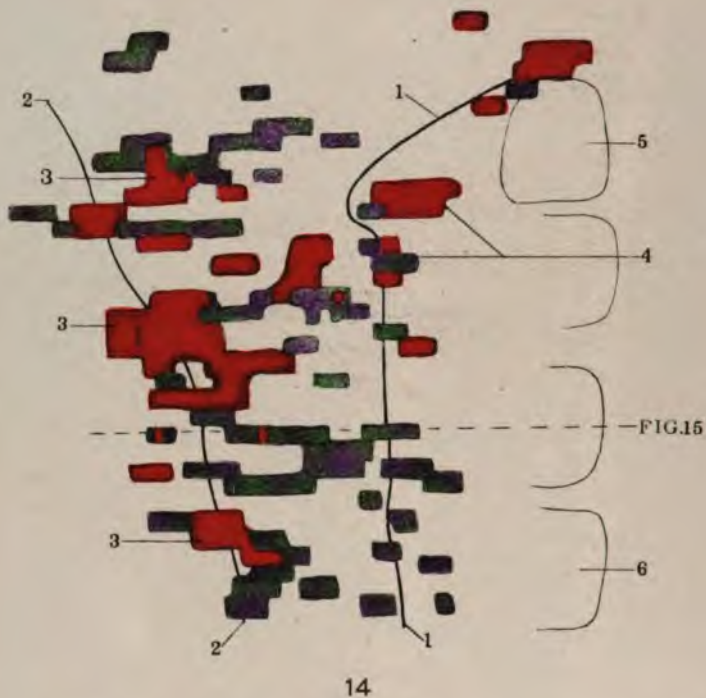


Fig. 14 Graphic reconstruction showing the distribution of the endothelium and vasofactive cells in the somatopleure of an embryo of twelve somites. The cavities are shown in red. Columbia Collection No. 540, left side, dorsal view, $\times 300$. Reduced $\frac{1}{4}$.

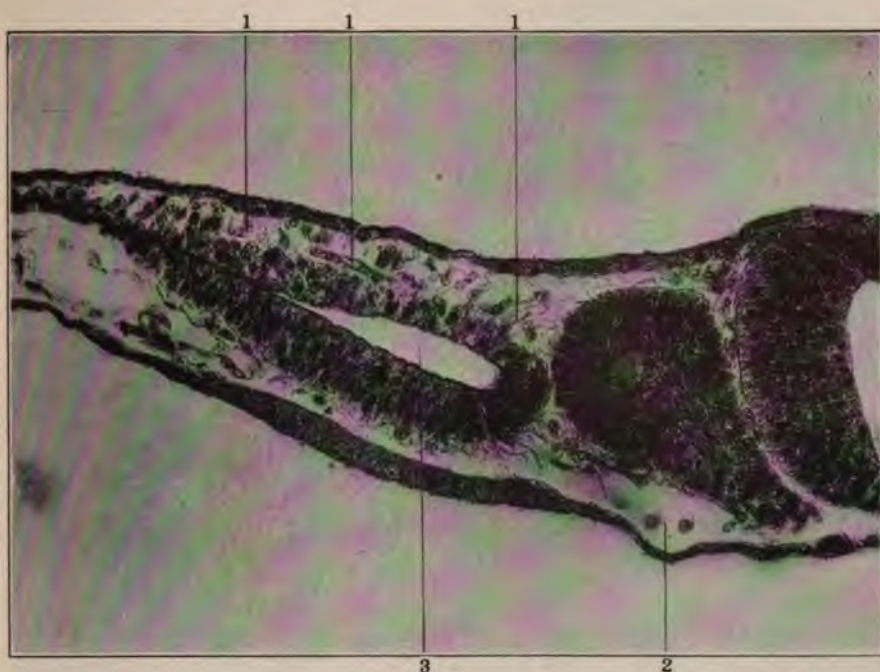
- | | |
|--------------------------------|-------------------------------|
| 1 Mesal margin of coelom | 4 Angiocysts of cardinal line |
| 2 Lateral margin of coelom | 5 Somite 1 |
| 3 Angiocysts of umbilical line | 6 Somite 4 |

Fig. 15 Transverse section of same embryo at level indicated in figure 14. Slide 3, row 2, section 10, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|---------------------|----------|
| 1 Vasofactive cells | 3 Coelom |
| 2 Aorta | |

Fig. 16 Graphic reconstruction of the vessels of the somatopleure and of a portion of the aorta from an embryo of fourteen somites. Columbia Collection No. 188, left side, dorsal view, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|---|--|
| 1 Umbilical vein | 5 Communication between the aorta and the umbilical vein |
| 2 Site of communication of the umbilical with the omphalomesenteric vein | 6 Aorta |
| 3 Anterior jugular vein | 7 Isolated angiocysts |
| 4 Communications of the anterior jugular vein with the juxta-neural anastomosis | 8 Somite 1 |
| | 9 Somite 4 |



15

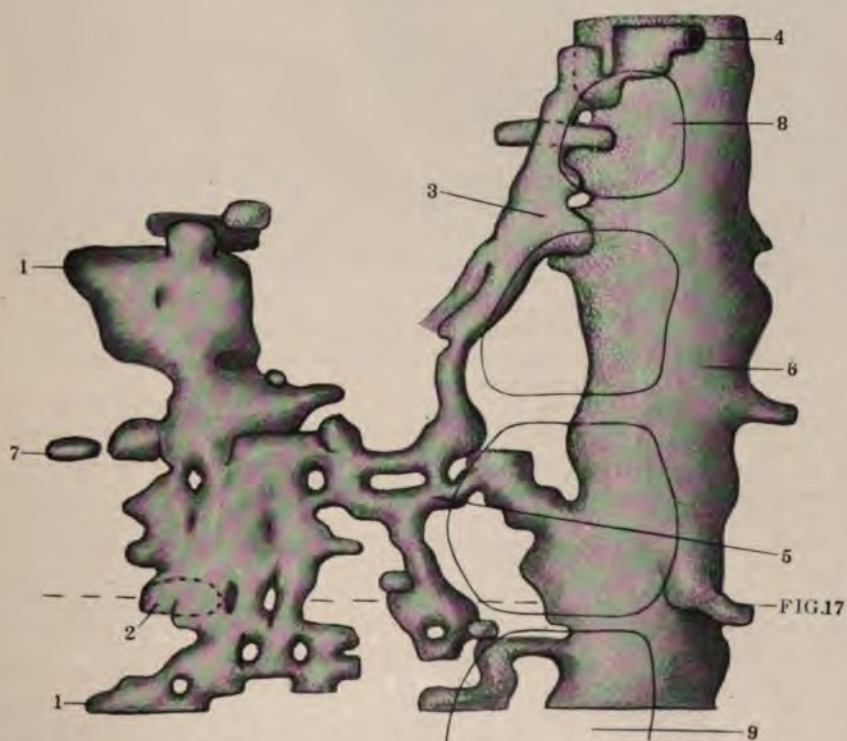


FIG.17

16

71

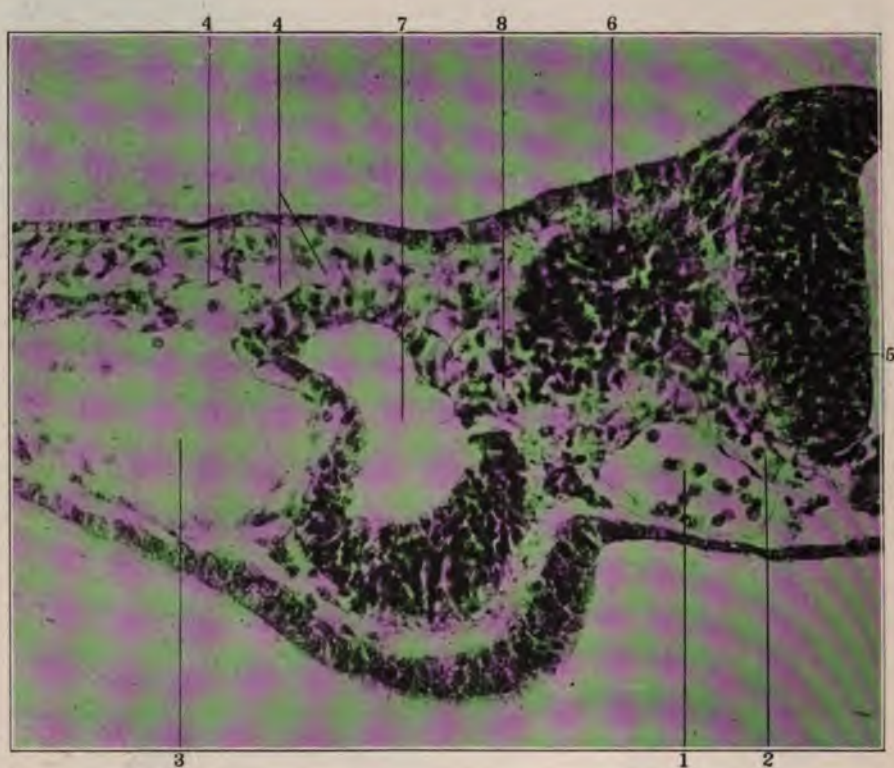


Fig. 17 Transverse section of the same embryo at level indicated in preceding figure, to show the communication between the umbilical and omphalomesenteric veins. Slide 4, row 4, section 6, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|---------------------------|--|
| 1 Aorta | 5 Juxta-neural vessel |
| 2 Dorsal segmental artery | 6 Somite 4 |
| 3 Omphalomesenteric vein | 7 Coelom |
| 4 Umbilical vein | 8 Section of vessel of cardinal position |



Fig. 18 Transverse section of an embryo of twelve somites to show the amniotic position of the caudal anlagen of the umbilical vein. Columbia Collection No. 547, slide 5, row 2, section 7, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|-----------------------|--------------------------------------|
| 1 Umbilical angiocyst | 4 Funnel-like diverticulum of coelom |
| 2 Amnion | between vessels of the splanchno- |
| 3 Mesostroma | pleure |



Fig. 19 Transverse section of an embryo of sixteen somites. Columbia Collection No. 551, slide 5, row 3, section 7, to show isolated endothelial vesicle in the amnion. $\times 230$. Reduced $\frac{1}{4}$.
1 Endothelial vesicle

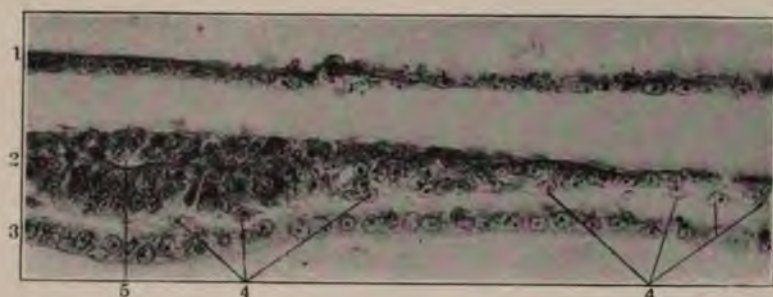
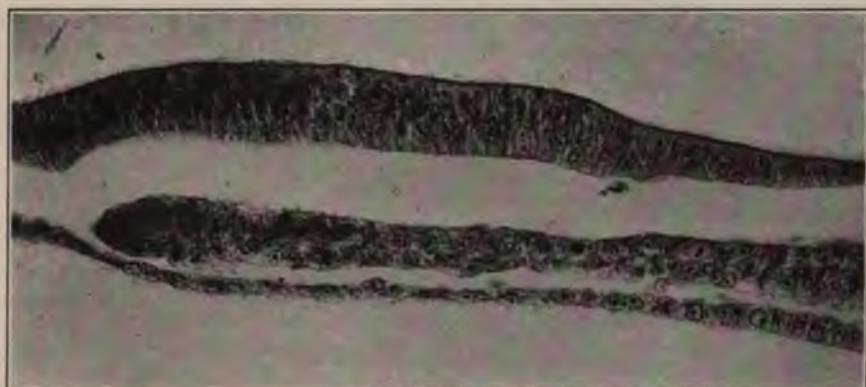


Fig. 20 Transverse section from embryo in which the somites are forming, but no complete intersomitic cleft has appeared. Columbia Collection No. 550, slide 3, row 4, section 6, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|------------|--|
| 1 Ectoderm | 4 Hyperentodermal mesenchyme and incipient blood islands |
| 2 Mesoderm | |
| 3 Entoderm | 5 Coelom |

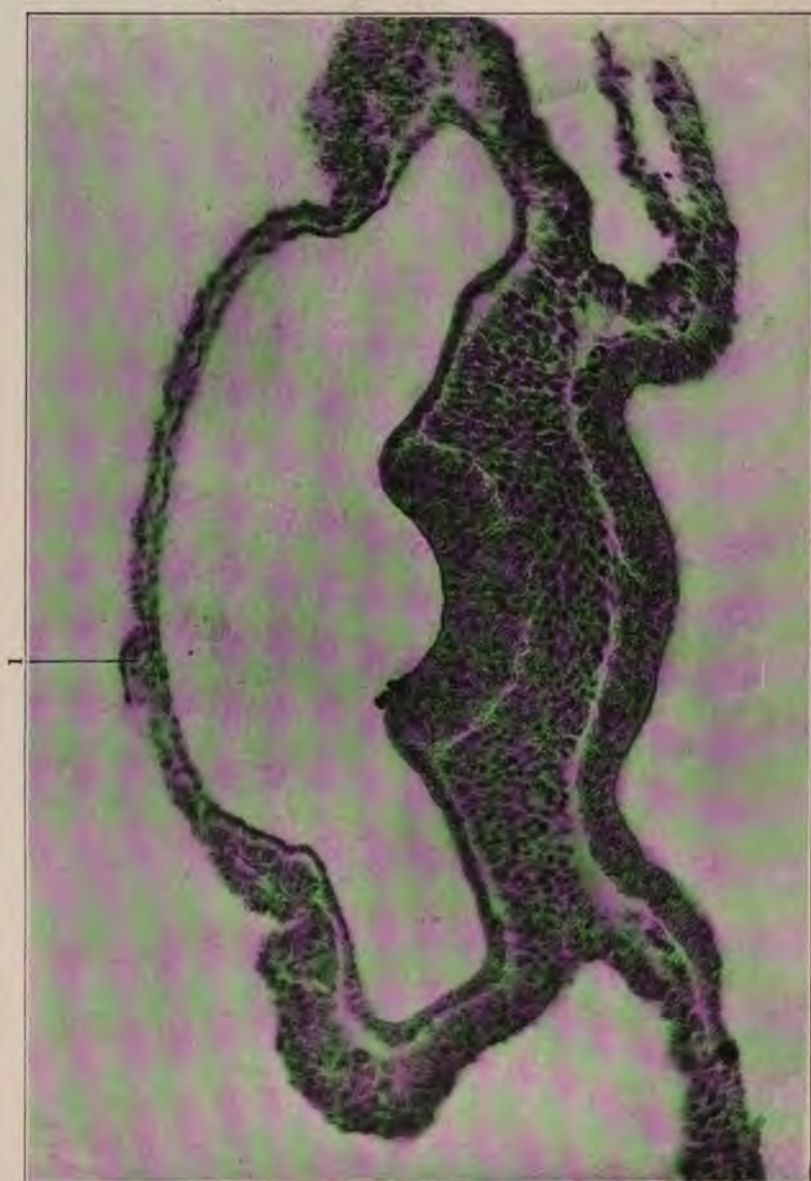


Fig. 19 Transverse section of an embryo of sixteen somites. Columbia Collection No. 551, slide 5, row 3, section 7, to show isolated endothelial vesicle in the amnion. $\times 230$. Reduced 4.
1 Endothelial vesicle

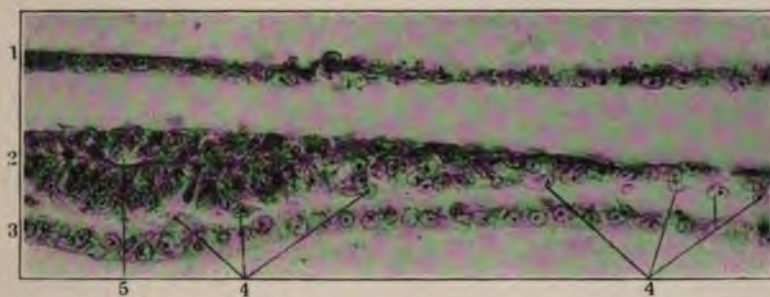
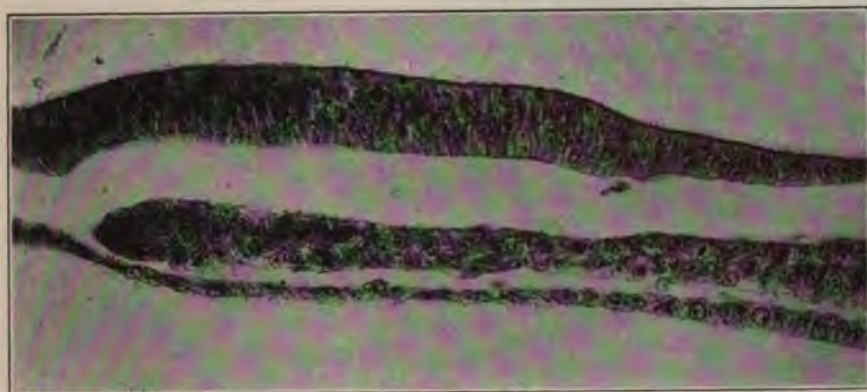


Fig. 20 Transverse section from embryo in which the somites are forming, but no complete intersomitic cleft has appeared. Columbia Collection No. 550, slide 3, row 4, section 6, $\times 300$. Reduced $\frac{1}{4}$.

- 1 Ectoderm
- 2 Mesoderm
- 3 Entoderm

- 4 Hyperentodermal mesenchyme and incipient blood islands
- 5 Coelom

Figs. 21 to 23 Ventral view of a model of the mesoderm (white), splanchnopleuric mesenchyme (green), vasofactive cells (yellow) and endothelium (red) of an embryo of two somites. Columbia Collection No. 539, $\times 300$. Reduced $\frac{1}{2}$.

- | | |
|--------------------------------------|---|
| 1 Anlages of ventral aorta and heart | 5 From this point craniad the mesoderm is continuous across the median line |
| 2 Anlages of dorsal aorta | |
| 3 First intersomitic cleft | |
| 4 Second intersomitic cleft | 6 From this point caudad the mesoderm is continuous across the median line |

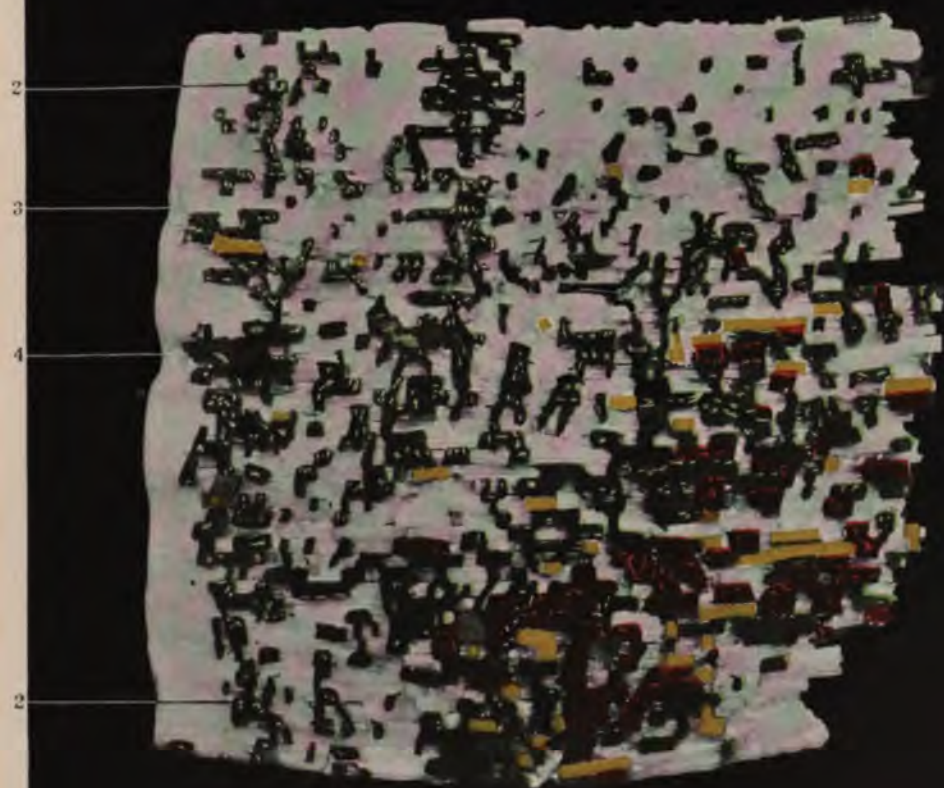
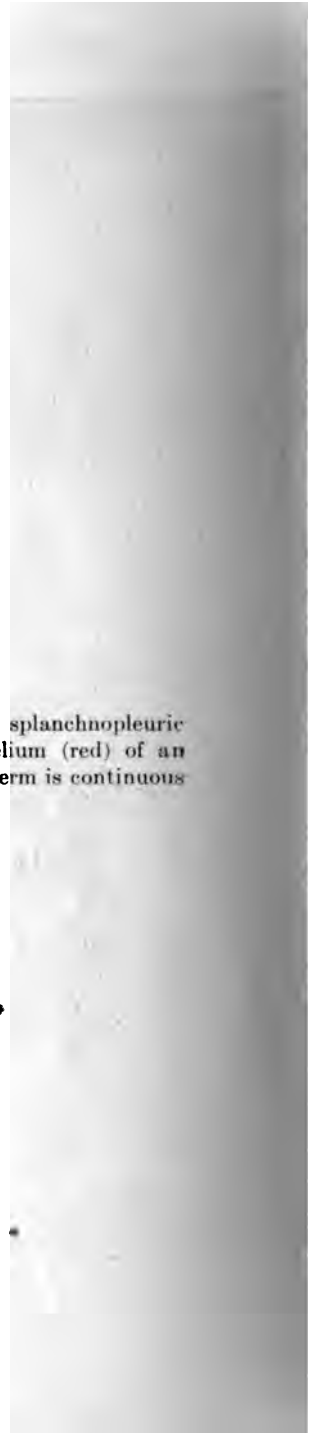


Fig. 23 Ventral view of a model of the mesoderm (white), splanchnopleuric mesenchyme (green), vasofactive cells (yellow) and endothelium (red) of an embryo of two somites. 6, From this point caudad the mesoderm is continuous across the median line.



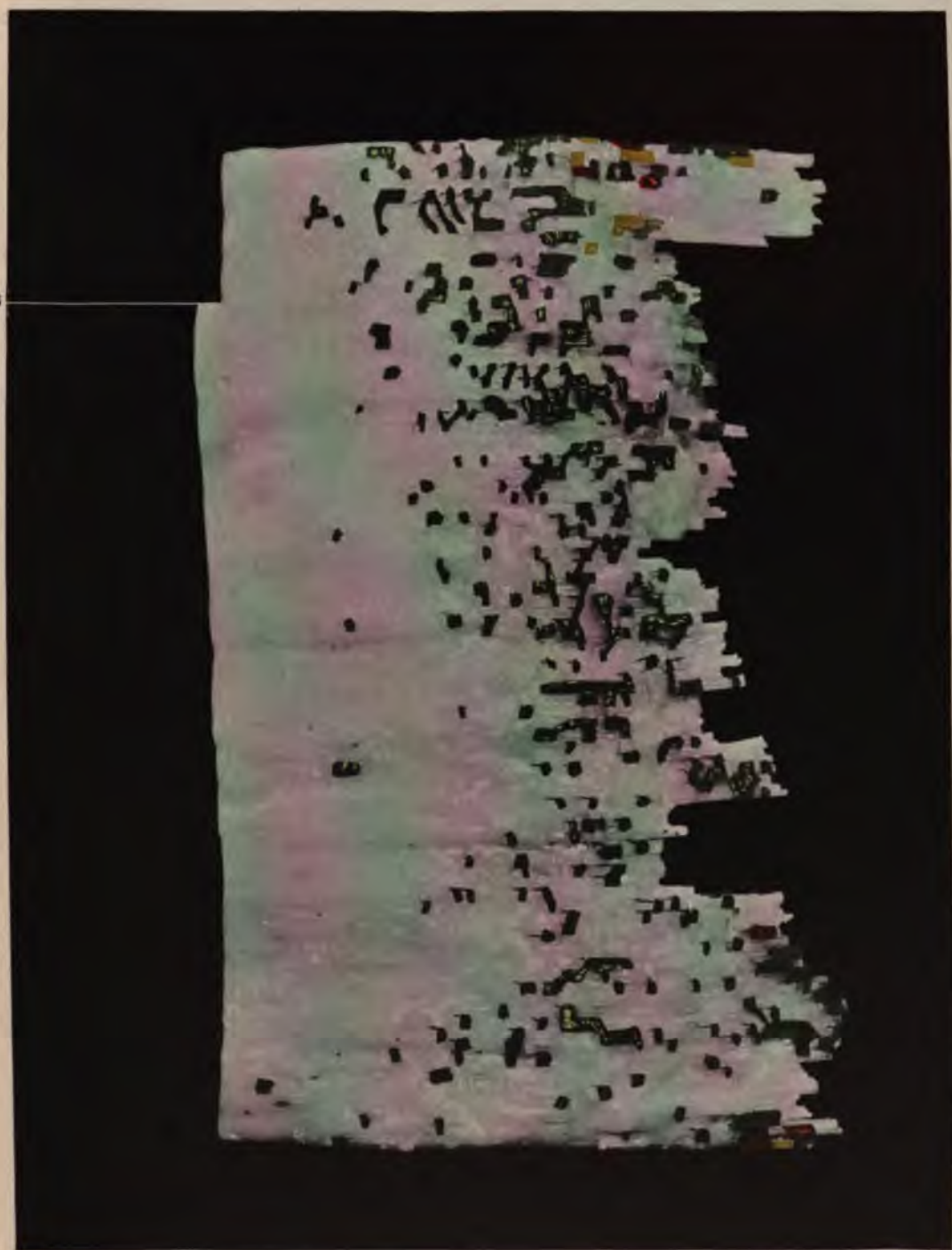
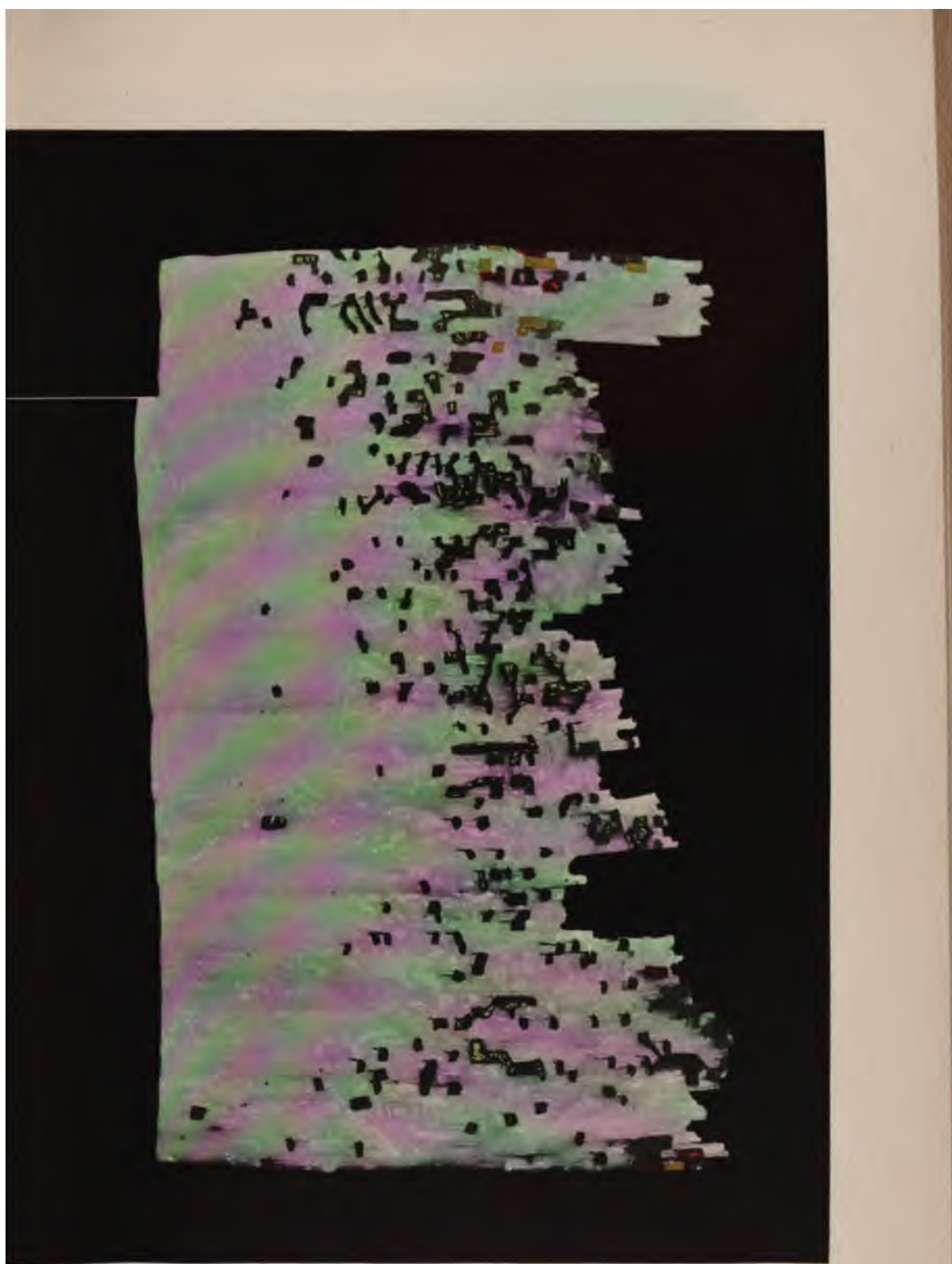




Fig. 23 Ventral view of a model of the mesoderm (white), splanchnopleuric mesenchyme (green), vasofactive cells (yellow) and endothelium (red) of an embryo of two somites. 6, From this point caudad the mesoderm is continuous across the median line.



23
81

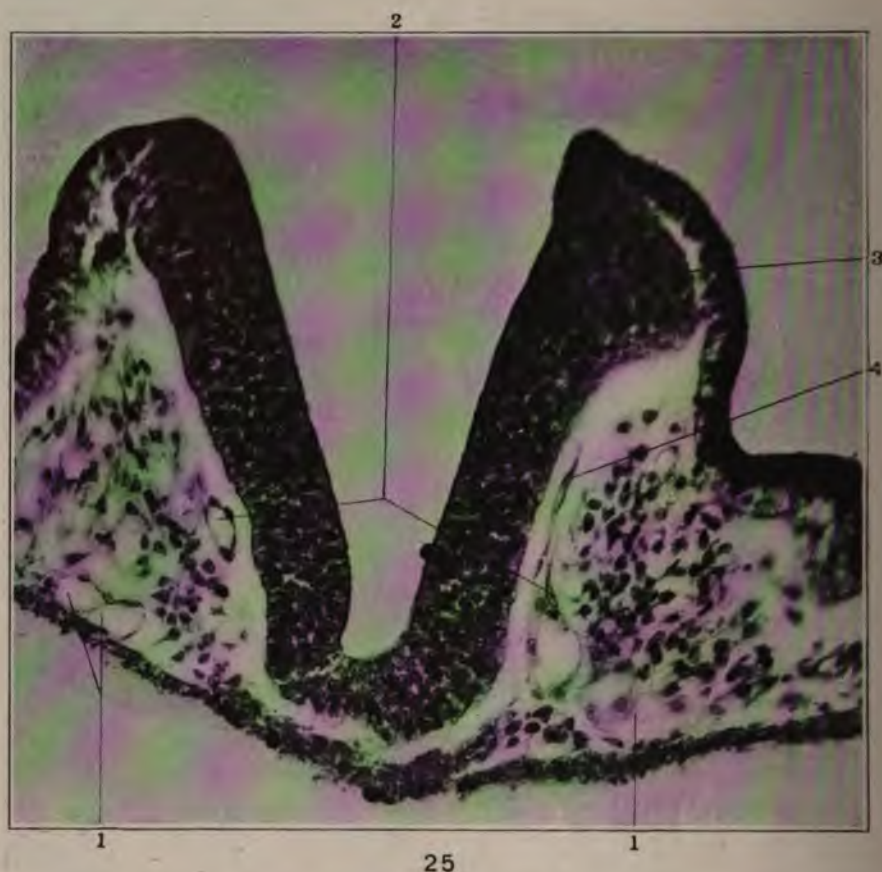
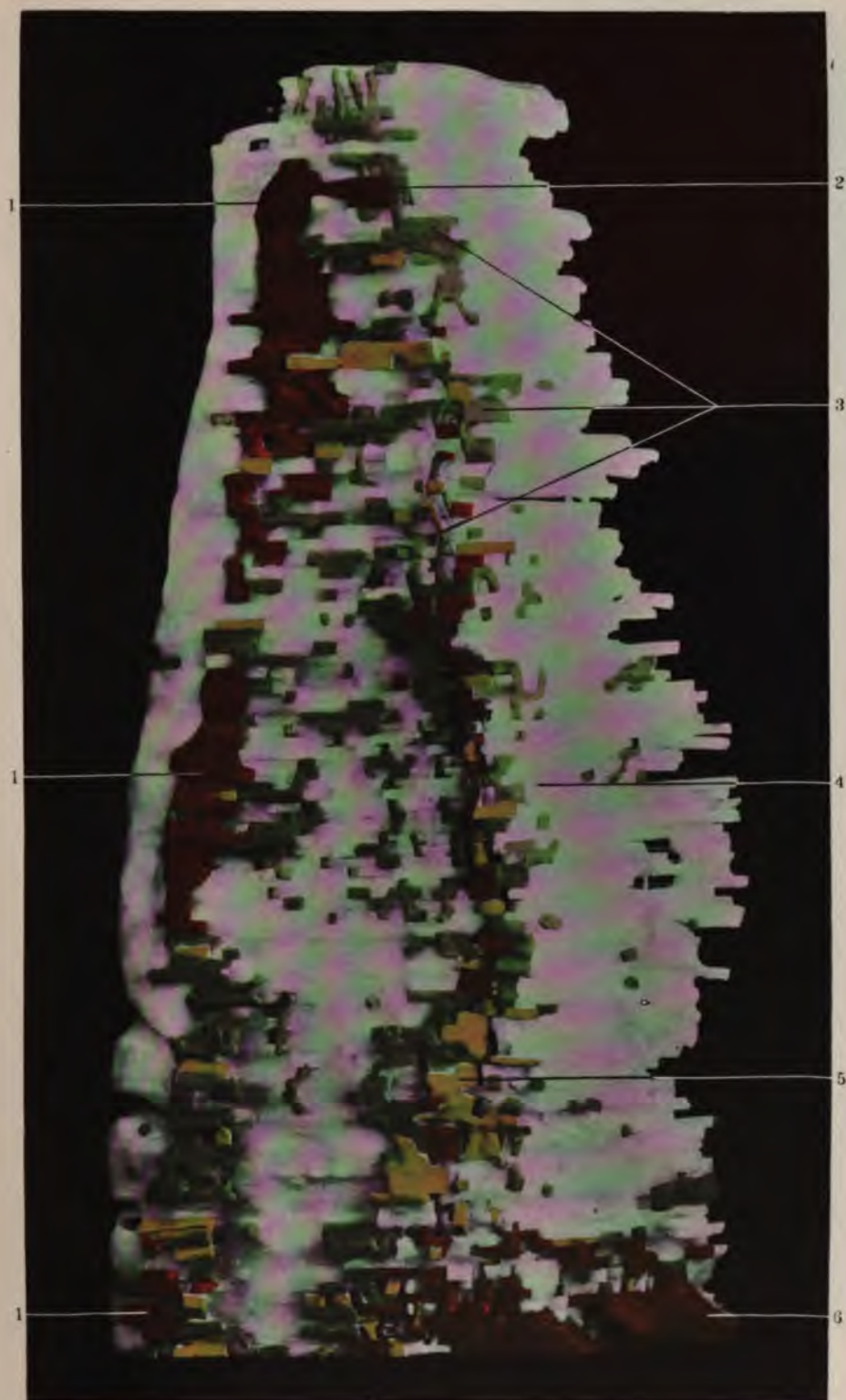


Fig. 25 Transverse section of an embryo of four somites at the level indicated by leader 3 in figure 24. Columbia Collection No. 409, slide 5, row 6, section 3, $\times 300$. Reduced $\frac{1}{4}$. The several angiocysts are in contact but their lumina are not continuous.

- | | |
|---------------------|--|
| 1 Aorta | 4 Vasofactive cells attached to juxta- |
| 2 Juxta-neural line | neural vessel; this cleft-like lu- |
| 3 Quintal anlage | men is not continuous with that |
| | of the vessel |

Fig. 26 Ventral view of a model of the mesoderm (white), mesenchyme (green), vasofactive cells (yellow) and endothelium (red) of an embryo of eight somites. Columbia Collection No. 530, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|----------------------------|------------------------------------|
| 1 Dorsal aorta | 4 Projections of visceral mesoderm |
| 2 Omphalomesenteric vein | which later become connected |
| 3 Omphalomesenteric plexus | with the aorta |
| | 5 Position of heart |



24
83

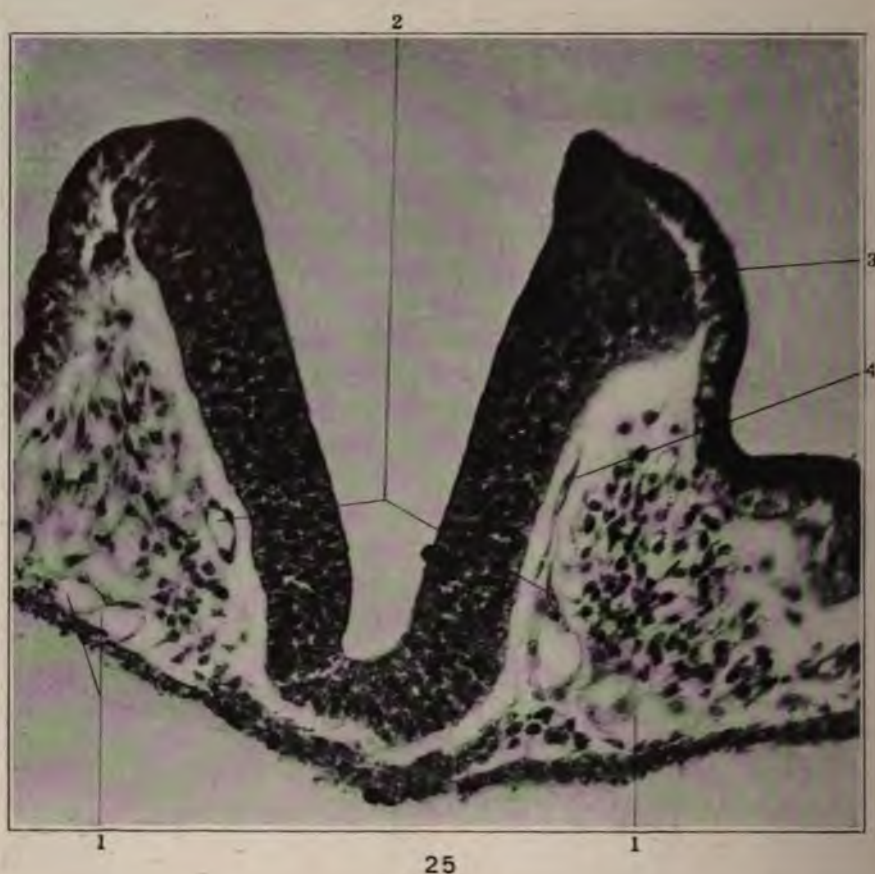
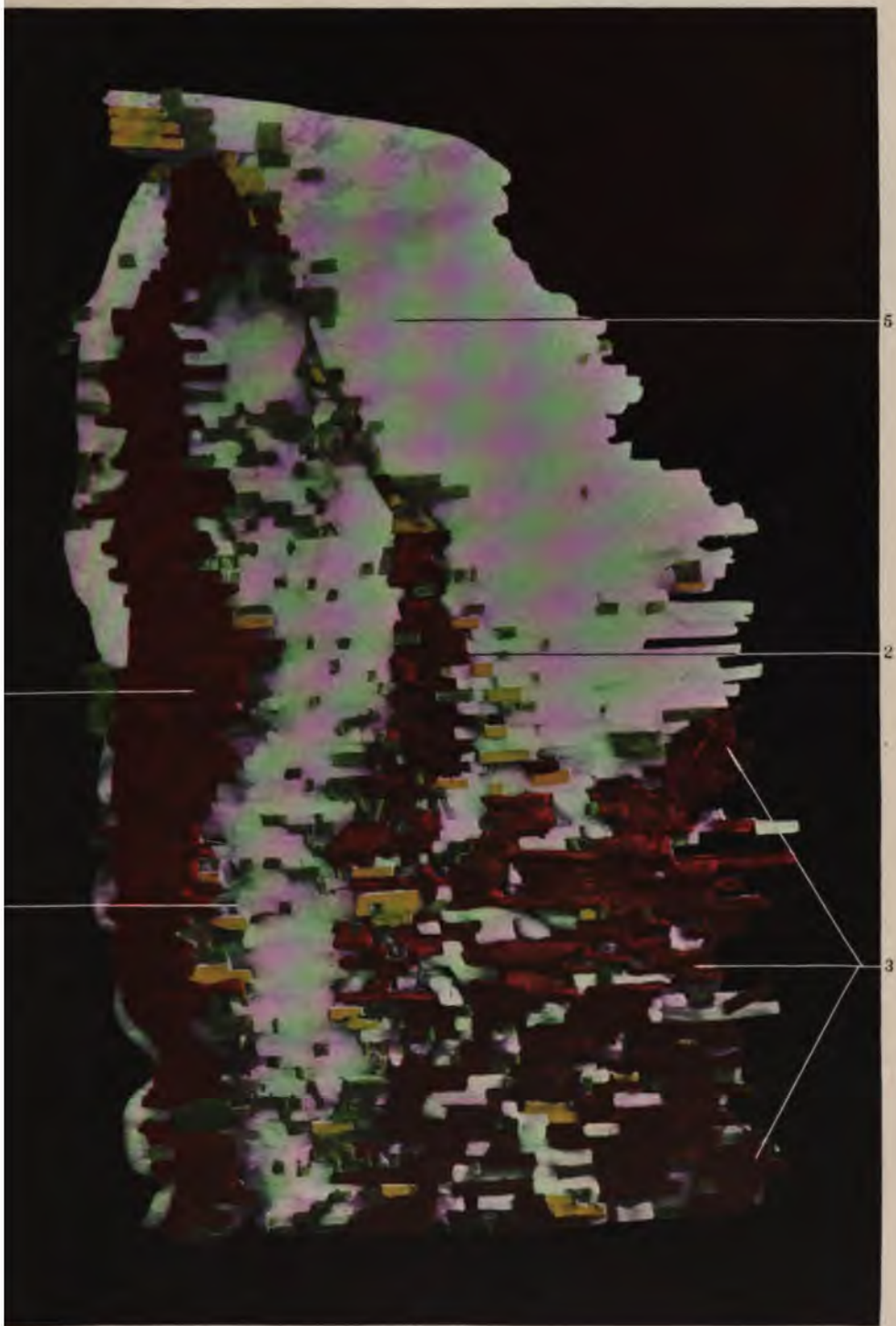


Fig. 25 Transverse section of an embryo of four somites at the level indicated by leader 3 in figure 24. Columbia Collection No. 409, slide 5, row 6, section 3, $\times 300$. Reduced $\frac{1}{2}$. The several angioecysts are in contact but their lumina are not continuous.

- | | |
|---------------------|--|
| 1 Aorta | 4 Vasofactive cells attached to juxta-neural vessel; this cleft-like lumen is not continuous with that of the vessel |
| 2 Juxta-neural line | |
| 3 Quintal anlage | |

Fig. 26 Ventral view of a model of the mesoderm (white), mesenchyme (green), vasofactive cells (yellow) and endothelium (red) of an embryo of eight somites. Columbia Collection No. 530, $\times 300$. Reduced $\frac{1}{2}$.

- | | |
|----------------------------|--|
| 1 Dorsal aorta | 4 Projections of visceral mesoderm which later become connected with the aorta |
| 2 Omphalomesenteric vein | 5 Position of heart |
| 3 Omphalomesenteric plexus | |



26
85

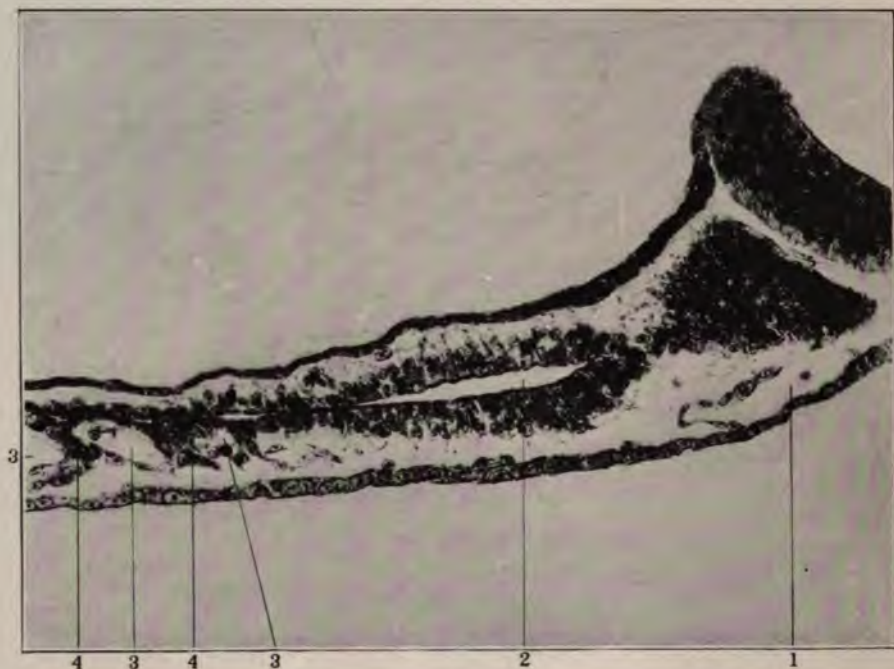
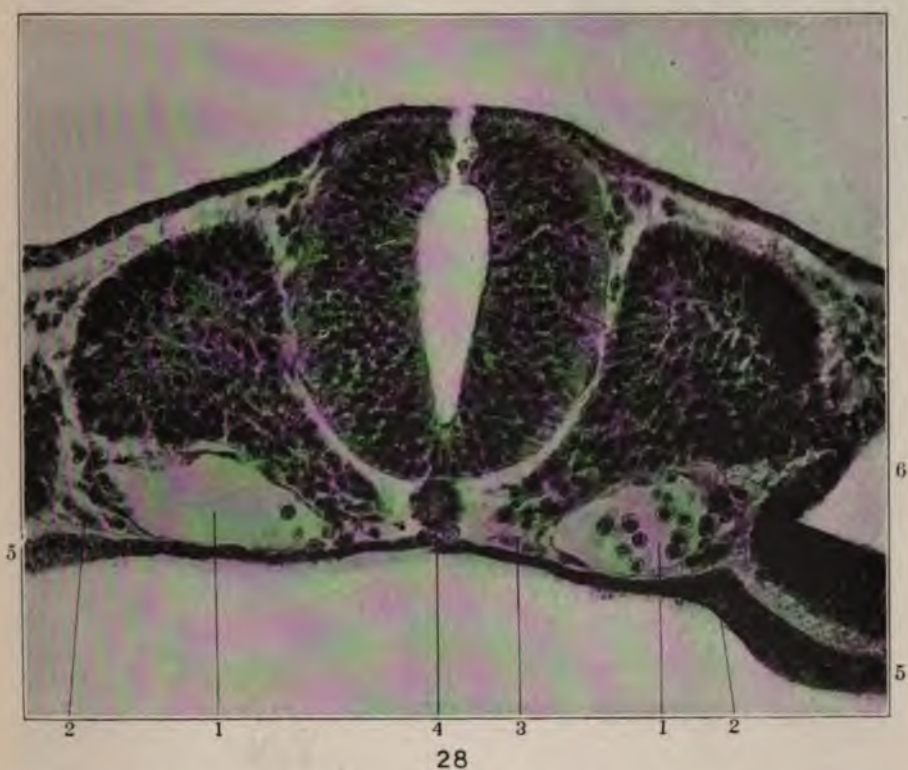


Fig. 27 Transverse section of an embryo of eight somites, Columbia Collection 530, slide 7, row 4, section 4, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|----------------------------|---|
| 1 Aorta | 4 Projections of mesoderm between blood vessels |
| 2 Coelom | |
| 3 Omphalomesenteric plexus | |



Figs. 28 to 33 Serial transverse sections of an embryo of twelve somites, Columbia Collection No. 547, slide 7, row 2, sections 7 to 12, $\times 300$. Reduced $\frac{1}{4}$.

- | | |
|---|--|
| 1 Aorta | 4 Notocord |
| 2 Cells derived from visceral mesoderm in process of accretion to the aorta | 5 Entoderm |
| 3 Cells of sclerotome in syncytial continuity with endothelium of aorta | 6 Coelom |
| | 7 Angiocyst added to aorta; a remnant of septum is still visible |



Fig. 29 Serial transverse section of an embryo of twelve somites. 2, Cells derived from visceral mesoderm in process of accretion to the aorta.



Fig. 30 Serial transverse section of an embryo of twelve somites. 2, Cells derived from visceral mesoderm in process of accretion to the aorta; 3, cells of sclerotome in syncytial continuity with endothelium; 7, angiocyst added to aorta; a remnant of septum is still visible.



Fig. 31. Serial transverse section of an embryo of twelve somites. 2, Cells derived from visceral mesoderm in process of accretion to aorta.

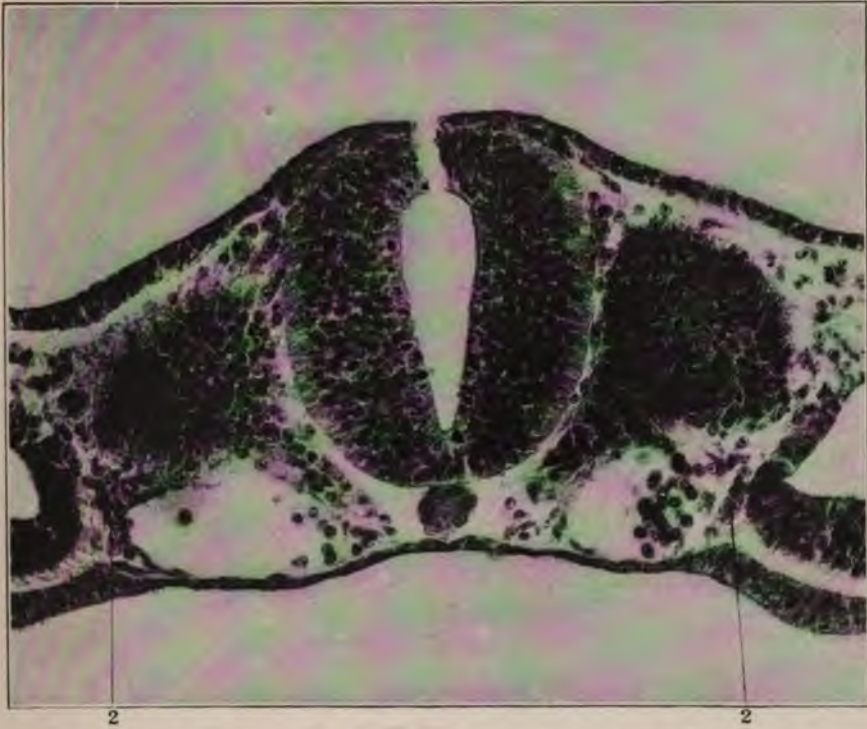


Fig. 32 Serial transverse section of an embryo of twelve somites. 2, Cells derived from visceral mesoderm in process of accretion to aorta.

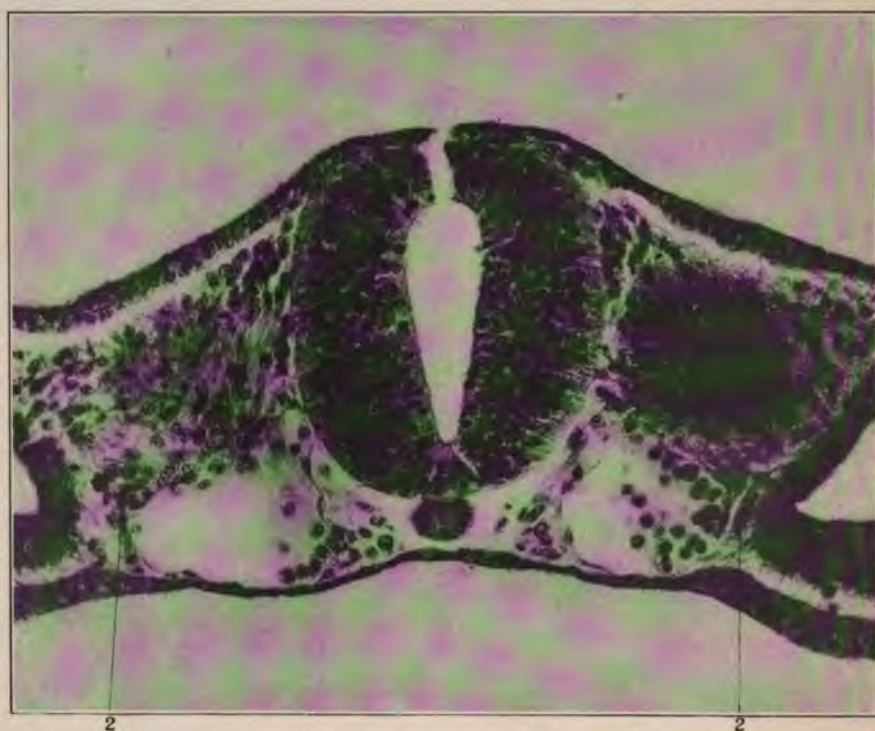


Fig. 33 Serial transverse section of an embryo of twelve somites. 2, Cells derived from visceral mesoderm in process of accretion to aorta.

MEMOIRS
OF
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
No. 4

THE DEVELOPMENT OF
THE LYMPHATIC SYSTEM IN FISHES
WITH ESPECIAL REFERENCE TO
ITS DEVELOPMENT IN THE TROUT¹

FORTY-TWO FIGURES

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PHILADELPHIA, PA.
1915

¹ Read before Section I of the Seventeenth International Congress of Medicine, London, August, 1913, and published in the Proceedings; also read before the American Association of Anatomists, December, 1913, and published in the Proceedings, Anat. Rec., vol. 8, 1914.

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I. INTRODUCTION

My studies on the development of the lymphatic system in fishes have thus far been confined to the vessels of the head and pharynx. These vessels were chosen for the reason that they have not been dealt with by others to any extent and because they present a greater variety of conditions for study than those situated in the trunk and tail regions. The fishes thus far studied consist of ganoids and teleosts and include *Amia calva*, *Lepidosteus osseus*, *Salvelinus fontinalis*, Mitchill (brook trout), *Salmo Gairdneri*, Richardson (steelhead trout) and *Salmo irideus*, Gibbons (rainbow trout). The vascular system of between six and seven hundred trout embryos has been injected² and the embryos studied during the process of injection, in transparent mounts after the method of Spalteholz, and in sections. Forty-two reconstructions have been made, after the method of Born, of the arteries, veins and lymphatics in the regions of the head and pharynx of *Amia*, *Lepidosteus* and the trout. These reconstructions form a fairly complete series which illustrate the development of the lymphatics from the time of their earliest appearance in the embryo, *as a series of discontinuous anlagen or lymph vesicles*, to the establishment of a condition in which a continuous system of lymph channels is present. Since the injection method has been employed in following the development of the lymphatics in the trout, I will deal, for the most part, with the conditions observed in this form.³

² The embryos were injected by my Assistant, Mr. Charles F. Silvester, to whom I am much indebted for his efficient services in this connection.

³ I wish to express my thanks and appreciation to Professor Raymond C. Osburn of the New York Aquarium and to Commissioner Hugh M. Smith of the U. S. Bureau of Fisheries, for their courtesy in supplying me with trout embryos.

II. THE EMBRYONIC GROUND-PLAN OF THE LYMPHATIC SYSTEM IN THE TROUT UPON WHICH THAT OF THE ADULT IS BUILT

The time at which the main lymphatic channels are first met with in the embryo in the form of a continuous system of vessels, naturally varies in relation to the temperature of the water in which development has taken place. It is therefore impossible to state definitely for any one of the species studied, that a system of continuous lymphatic channels will be uniformly met with on any particular day. In the rainbow and steelhead trout, when developed at an average temperature of about 10.5°C., a continuous system of lymphatic channels was found to be present on both sides of the head and pharynx on the twenty-second day after fertilization in fully 90 per cent, and, on the twenty-third day, in practically all of the embryos injected. In another series of steelhead trout embryos developed at an average temperature of 11.5°C, a continuous system of lymphatic channels was met with on both sides of the embryo on the twentieth day after fertilization in practically all of the embryos injected.

Figure 10 represents a reconstruction of the principal arteries, veins and lymphatics (main trunk lines) found in the regions of the head and pharynx of a rainbow trout embryo on the twenty-second day after fertilization. It represents the earliest condition met with in the trout embryo in which the principal or main lymphatic channels of the head and pharynx form, for the most part, a continuous system of vessels which drain into the veins at typical points. *This particular reconstruction may be said to represent the ground-plan arrangement of the embryonic lymphatic system upon which that of the adult trout is built.* It was reconstructed from sections of an uninjected embryo in order to emphasize the ease and accuracy with which the lumina of the lymphatics can be followed in sections, without the aid of injections, after continuous vessels have been established. The principal lymphatic channels are as follows:

1. The lateral pharyngeal lymphatic.
2. The subocular lymph sacs.
3. The medial pharyngeal lymphatic.
4. The precardinal or jugular lymphatics.

1. *The lateral pharyngeal lymphatic (3 in figure 10, truncus lymphaticus pharyngeus lateralis)*

The lateral pharyngeal lymphatic occupies a superficial position in the lateral wall of the pharynx and forms, on each side of the body, a direct anterior continuation of the lymphatic of the lateral line of the trunk (*truncus lymphaticus longitudinalis lateralis*, 10 in fig. 10). The lateral pharyngeal lymphatic drains the subocular lymph sac, the dorsal region of the head and pharynx and, at a slightly later stage, the operculum and the lower jaw. In fact, with the exception of one other channel (medial pharyngeal lymphatic), the lateral pharyngeal lymphatic and its tributaries, together with the subocular lymph sac, constitute, on each side of the body, the entire continuous lymphatic system found in the regions of the head and pharynx at this stage of development. The lymphatic of the lateral line of the trunk (10, fig. 10) is also completely developed on the twenty-second day and can be traced caudad into the tail region.

The points of communication established between the lateral pharyngeal lymphatic (3) and the precardinal (jugular) vein (6) are confined, at this period of development, to that portion of the precardinal vein which extends between the caudal end of the otocyst and the point where the pre- and postcardinal veins (6, 11) unite to form the duct of Cuvier (24, cardino-Cuvierian junction). A point of communication commonly occurs at the cardino-Cuvierian junction (cardino-Cuvierian communication, 9 in fig. 10) where the lateral pharyngeal lymphatic (3) opens into the veins in common with the lymphatic of the lateral line of the trunk (10). This communication may be single or multiple in character, the latter being the case on both sides of the embryo under consideration. Another point of communication with the precardinal commonly occurs at about opposite the caudal end of the otocyst (otic communication) and in the embryo under consideration, was present only on the right side (13 in fig. 10). The otic communication may be present on both sides of the embryo, however, and, like that found at the cardino-

Cuvierian junction, may be either single or multiple in character. In addition to these more typical points of communication, the lateral pharyngeal lymphatic may also communicate with the precardinal at almost any point between the two; so that the region of the precardinal vein which lies between the otic (13) and cardino-Cuvierian communications (9) may be regarded, in general, as constituting a district of lymphatico-venous communication.

2. *The subocular lymph sacs (1 in figure 10, saccus lymphaticus subocularis)*

The subocular lymph sacs consist of two relatively huge sacs, each of which is situated ventro-medially to the eye. They are more or less triangular in form, with their apices directed anteriorly. In the twenty-two-day trout under consideration, they extend between the hyoidean artery (15) and the olfactory pit (33 in fig. 14). They do not communicate with each other nor with the veins in the immediate neighborhood of the sacs. At its posterior lateral angle, each subocular lymph sac communicates directly with the lateral pharyngeal lymphatic (3) of its respective side and drains through the latter into the veins at the typical points of communication, mentioned above, which the lateral pharyngeal lymphatic establishes with the veins (9 and 13 in fig. 10).

3. *The medial pharyngeal lymphatic (4 in figure 10, truncus lymphaticus pharyngeus medialis)*

This vessel occupies a more medial position and is more deeply situated than the lateral pharyngeal lymphatic. It runs an oblique course, in a postero-anterior direction, from about the middle of the lateral pharyngeal lymphatic, with which it subsequently establishes a communication, to open into the precardinal (jugular) vein just caudal to the point where the latter emerges from the cranial cavity. This communication (medial pharyngeal communication, 2 in fig. 10), together with those

mentioned above (9 and 13), constitute all of the typical points of communication, as far as I can determine, which are established in the trout embryo, in the regions of the head and pharynx, between the lymphatics and the veins.

4. *The precardinal or jugular lymphatics (5 and 5' in figure 10, truncus lymphaticus precardinalis vel jugularis)*

The precardinal lymphatics are not completely established in the form of a continuous system of channels on the twenty-second day. They follow the course of the precardinal veins and, in the twenty-two-day rainbow trout embryo, are represented by several independent and discontinuous lymph vesicles or sacs (5' in fig. 10) and by two irregularly-shaped sacs (5 in fig. 10). The latter lie in the region of the three posterior efferent aortic arches (17, 18 and 19, fig. 10), and communicate with the lateral pharyngeal lymphatic about opposite the caudal end of the otocyst (7 in fig. 10). At a slightly later stage of development the precardinal lymphatics form a continuous system of vessels which drain into the veins through the lateral and medial pharyngeal lymphatics.

The above constitute the main or principal continuous lymphatic channels which are found in the steelhead and rainbow trout embryo on the twenty-second day after fertilization, and also in the brook trout at a corresponding stage.

If one injects into the caudal vein at this stage of development, on account of the absence of lymphatico-venous valves, the injecta will pass freely into the lymphatics from the veins through any one or all of the typical points of communication which exist between the two. It will therefore fill up completely, on each side of the body, the lateral pharyngeal lymphatic, the subocular lymph sac, the lymphatic of the lateral line of the trunk, the medial pharyngeal lymphatic and that portion of the precardinal lymphatic (5) which has established a communication with the lateral pharyngeal lymphatic near the caudal end of the otocyst. The injecta will not pass into the independent

and discontinuous anlagen of the precardinal lymphatics (5') since these do not as yet communicate with the continuous lymphatic system as a whole. The above-mentioned continuous lymphatic system, as well as the veins, can also be readily injected from the subocular lymph sacs.

Observations made upon the living trout embryo at this stage of development have also shown that, in the absence of lymphatico-venous valves, blood may pass from the veins into the lymphatics. In a great number of instances blood was observed to pass from the precardinal vein into the lateral pharyngeal lymphatic of a living trout embryo and, after filling completely the subocular lymph sac, to flow back into the veins. Figure 15 is a section of a twenty-day steelhead trout embryo which passes through both subocular lymph sacs and in which it is seen that the sac on the right side is completely filled with blood, while that on the left side is empty. In all such cases as those mentioned above, the blood-filled subocular lymph sac has invariably established its connection with the lateral pharyngeal lymphatic and, in the case of the twenty-day steelhead trout embryo (fig. 15), this connection was established only upon the right side. In trout embryos older than twenty-two days, and in which lymphatico-venous valves have been formed, the application of chloretone was found to vitiate the normal function of the valves. Blood would even then flow freely into the lymphatics from the veins and fill up completely all of the lymphatic channels, including the subocular lymph sacs, which had established a communication with the veins. When such embryos were removed to water in which no chloretone was present, the blood usually flowed back into the veins from the lymphatics. These so-called blood-injected embryos proved of great service in many instances, in following the course of lymphatic channels. In abnormal living trout embryos, especially those with spinal curvature, and at an age at which lymphatico-venous valves should be present, blood was frequently found in the subocular lymph sac and in the lateral pharyngeal lymphatic.

There is no question whatever that venous blood may be present in the lymphatics of the living trout embryo during certain stages of development. The only possible question at issue is the significance which the presence of venous blood in the lymphatics implies.

III. ARE THE LYMPHATICS OF FISHES TRANSFORMED VEINS?

The presence of venous blood in the lymphatics has given rise to the view that the lymphatics of fishes are transformed veins and that they are more closely related to the veins than the lymphatics of the higher vertebrates. This view, I think, was originally advanced by Favaro ('06). More recently Mőzejko ('13) has advocated the view that a true lymphatic system, similar to that found in mammals, is not even found in fishes but is met with for the first time in the amphibia.

If the view that the lymphatics of fishes are transformed veins signifies that certain vessels of the embryo which primarily function as veins subsequently assume a lymphatic function, I have positive proof, I believe, in the case of the subocular lymph sacs of the trout, that such is not the case and proof to this effect will, as we shall see, similarly disprove the contention of Mőzejko. On the other hand, if the "transformation" theory merely signifies that the lymphatics of fishes are derived from the endothelium of the veins, that is another question and one which forms the main theme of this paper. As I interpret the "transformation" theory, it signifies that vessels which function primarily as veins may subsequently assume a lymphatic function.

If the lymphatics of fishes are transformed veins and are more closely related to the veins than the lymphatics of the higher vertebrates, they should invariably function in the capacity of veins, or, at least in the capacity of both veins and lymphatics, at the time of their first appearance in the embryo. This is positively not the case, however, with the subocular lymph sacs of the trout embryo and detailed evidence to this effect will be presented in the following pages. It may be stated here that during the earliest stages of their development the subocular

lymph sacs of the trout embryo function entirely independently of the veins and serve solely as independent reservoirs for the reception of lymph which they receive from the intercellular tissue spaces of the head region. At a later stage of development, however, these sacs then secondarily establish a communication with the veins through another lymphatic (lateral pharyngeal lymphatic, 3 in fig. 10) and, at such a time, and then only before lymphatico-venous valves have been formed, can the subocular lymph sacs of the trout receive venous blood from the veins. In view of these observations, those who maintain that the presence of venous blood in the lymphatics of fishes indicates that their lymphatics are transformed veins, or, contend that fishes do not possess a lymphatic system which is comparable to that of other vertebrates, will have to explain why the subocular lymph sacs of the trout embryo first function solely as lymphatics, and may then assume a so-called venous function, only after they have established a secondary and indirect connection with the veins.

As previously stated by Huntington and McClure ('10), I believe that the lymphatics, as distinguished from the veins, are lymphatics from the time of their first appearance in the embryo. My observations on the trout embryo lead me to believe that the passage of venous blood from the veins into the lymphatics is a procedure of no physiological significance whatever and, under normal conditions, is alone made possible by the absence of lymphatico-venous valves. In ordinary circumstances and before lymphatico-venous valves have been formed in the trout embryo, the flow of venous blood into the lymphatics may possibly be related to an intermittent flow of lymph into the veins. In many cases observed in the living trout embryo it appeared to be largely accidental and could be induced by a rough handling of the embryo, by the application of chloretone, or by the shock produced at the time the embryo was placed in the fixing agent. In the absence of lymphatico-venous valves there is no physical impediment to prevent the blood from passing into the lymphatics from the veins. After these valves have been formed, however, it is extremely difficult to force the injecta into the

lymphatics from the veins without causing extravasations, and I therefore doubt if the flow of blood through valves which *are especially constructed to prevent its passage can be regarded at any time, either in fishes or in any other vertebrate, as a normal procedure.* It is true that blood corpuscles are often found in the lymphatics of the trout after the lymphatico-venous valves have been established. In such cases, however, if these corpuscles have not been forced through the valves under unusual conditions, it is likely that they are either the remains of those which passed into the lymphatics before valves were formed, or corpuscles which the lymphatics have taken up from the tissue spaces.

Although I have not had the opportunity of studying the living embryos of *Amia* and *Lepidosteus*, I can state from a study of sections that, before lymphatico-venous valves have been formed, venous blood flows into the lymphatics from the veins. After the formation of valves, however, the blood no longer has free access to the lymphatics and, in this respect, the conditions found in the embryos of ganoids are the same as those in the trout.

It must be borne in mind that the passage of blood from the veins into the lymphatics is a phenomenon *not exclusively confined to the embryos of fishes.* In the embryos of all other vertebrates, including mammals, blood must necessarily have free access to the lymphatics during those early stages of development in which lymphatico-venous valves are wanting and in which the lymphatics are capable of being freely injected from the veins. Since the passage of venous blood into the lymphatics normally ceases with the formation of lymphatico-venous valves, it is hardly probable that its presence in the lymphatics of the embryo, in either fish or mammal, is indicative of any physiological condition which is essential to the economy of the vascular system at a time before, but not after lymphatico-venous valves have been formed. It is quite possible in the case of some fishes that lymphatico-venous valves may never be formed. Even in such cases, I believe that the influx of venous blood into the lymphatics would possess no physiological significance beyond that temporarily displayed in the embryos of vertebrates in gen-

eral, at a time before lymphatico-venous valves have been formed. This influx we have seen is largely accidental in trout embryos and, even then, is practically only permissible in the absence of lymphatico-venous valves. In view of what has been stated above regarding the subocular lymph sacs of the trout, and the presence of blood in the lymphatics of vertebrates in general (in relation to the absence of lymphatico-venous valves), it is plain that the presence of blood in the lymphatics in no sense indicates that the lymphatics of fishes are transformed veins, or, that they are more closely related to the veins than the lymphatics of other vertebrates.

Miller ('13) and Huntington ('14) have applied the term 'haemophoric' to certain lymphatics of amniotes which convey blood corpuscles developed in the adjacent mesenchyme to the venous circulation. As far as I have been able to determine haematopoiesis does not occur in the trout and ganoids in relation to the lymphatics of the head and pharynx. The influx of blood from the veins into the lymphatics of fishes is, therefore, in no sense comparable to what Huntington and Miller have described as the 'haemophoric' function of the lymphatics in the embryos of amniotes.

IV. THE MODIFICATIONS WHICH THE EMBRYONIC GROUND-PLAN OF THE LYMPHATIC SYSTEM UNDERGOES IN ESTABLISHING THE MORE ADVANCED STAGES IN THE TROUT

The lymphatic system as found in the head and pharynx of the twenty-two-day rainbow trout (fig. 10) represents a ground-plan upon which that of the adult is necessarily built. I have not followed in detail all of the transformations which lead up to the adult condition, but have examined and reconstructed several advanced stages in which the conditions cannot, in the main, be unlike those found in the adult, and which are easily interpreted on the basis of their origin from such a ground-plan.

The modifications which this primary ground-plan undergoes in subsequent stages of development are largely related: (1) To the increasing importance gradually attained by the precardinal lymphatics and their tributaries; (2) to the apparently complete

detachment and separation of the subocular lymph sacs from the rest of the lymphatic system, and (3) to the forward extension of the lateral pharyngeal lymphatic into the head region as the *superficial facial lymphatic*, which aids in draining this region in place of the subocular lymph sac. These modifications are shown in figure 11 which represents a reconstruction of the principal lymphatics, veins and arteries found on the left side of the head and pharynx in a 17.5 mm. brook trout embryo.

In the 17.5 mm. brook trout embryo (fig. 11) the precardial lymphatics (5) form a much more extensive system of vessels than in the rainbow trout on the twenty-second day (fig. 10). They drain the gill arches, the walls of the pericardial cavity and the dorsal mesentery, and communicate with venous circulation through the medial and lateral pharyngeal lymphatics. As shown by the reconstruction, the lateral pharyngeal lymphatic (3) of the left side has lost its original connection with the lymphatic of the lateral line of the trunk (10) in the 17.5 mm. brook trout embryo. The result is that the lymphatic drainage of the head and pharynx is now independent of that of the lymphatic of the lateral line of the trunk and enters the venous circulation only by way of the medial pharyngeal communication (2) and, to a lesser extent, at a point near the caudal end of the otocyst, where the lymphatics also communicate with the veins (13, otic communication). The medial pharyngeal communication appears to be retained in still later stages and in the adult trout, as the principal point at which the lymphatic drainage of the head and pharynx enters the venous circulation.

It has been stated above that the left lateral pharyngeal lymphatic has lost its connection with the lymphatic of the lateral line of the trunk and, in this 17.5 mm. brook trout embryo, there seems to be no doubt that such is the case. I have evidence, however, which seems to show that the lateral pharyngeal lymphatic may not always establish a connection with the veins on both sides of the embryo, either at the cardino-Cuvierian junction, or near the caudal end of the otocyst (otic communication). It is evident, therefore, that variations found in advanced stages cannot always be attributed to the loss of com-

munications which formerly existed in the embryo, since certain of these communications may never have been formed at the typical points. Such a condition is shown in the twenty-two day rainbow trout embryo (fig. 10) in which the otic communication (13) is present only on the right side. In general, such variations as those mentioned above would fall in line with those observed in mammals (Huntington and McClure '08; and McClure and Silvester '09). Here we know that the points of communication which the jugular lymph sacs establish with the veins fall within two typical districts of communication (jugulo-subclavian and common jugular angles), and that communications may be established within only one of the two or within both of these districts.

The communication established between the subocular lymph sac and the lateral pharyngeal lymphatic on the twenty-second day (fig. 10) is, as far as I can determine, not permanently retained in quite advanced embryonic stages and in the adult. At a certain period after hatching the subocular lymph sac becomes detached from the lateral pharyngeal lymphatic so that the sac ceases to drain through the latter into the veins. Also, after its detachment, the subocular lymph sac of the trout appears to remain detached from the lateral pharyngeal lymphatic during all subsequent stages of development, and neither establishes any further communication with the veins, nor with any other portion of the lymphatic system. In correlation with the loss of the subocular lymph sac as an organic part of a connected lymphatic system, a new lymphatic channel makes its appearance in the embryo, *the superficial facial lymphatic*, which aids in draining the region of the head formerly drained by the subocular lymph sac. The superficial facial lymphatic (21), as shown in the 17.5 mm. brook trout (fig. 11), forms the direct anterior continuation in the head region of the lateral pharyngeal lymphatic (3).

It is difficult to establish definitely for the three species of trout embryos studied, the exact time at which the detachment and separation of the subocular lymph sac from the lateral pharyngeal lymphatic occurs. The sacs were invariably found to be de-

tached in the more advanced embryonic stages, as in the 17.5 mm. brook trout (fig. 11), and as early as in an 18.25 mm. rainbow trout embryo. On treating an 18.25 mm. rainbow trout embryo with chloretone the lateral pharyngeal lymphatic and its anterior continuation in the head region, the superficial facial lymphatic, rapidly filled with blood. A section taken through the caudal end of the subocular lymph sac of this embryo (fig. 16) shows that a partition has been formed between the sac (1) and the lateral pharyngeal lymphatic (3) which now prevents the blood from passing from the latter into the sac. A section (fig. 17) taken through the same embryo, somewhat anterior to the level of the former section, shows the independent character of the blood-filled superficial facial lymphatic (21), as well as its relation in the head region to the subocular lymph sac (1).

V. THE EMBRYONIC GROUND-PLAN OF THE LYMPHATIC SYSTEM
IN GANOIDS AND THE SUBSEQUENT TRANSFORMATIONS WHICH IT UNDERGOES

The embryonic ground-plan of the lymphatic system is exactly the same in ganoids as that described above for the twenty-two-day rainbow trout (fig. 10). In *Amia* and *Lepidosteus*, as in the trout, the subocular lymph sacs, the lateral and medial pharyngeal lymphatics, together with the precardinal lymphatics, constitute the framework upon which the structure found in the later stages of the embryo and in the adult is necessarily built. I have paid more attention to *Amia* than to *Lepidosteus* and will therefore confine my remarks, for the most part, to the conditions observed in this form.

In *Amia* and *Lepidosteus* each subocular lymph sac (1) early establishes a direct communication with the precardinal vein in the *immediate* neighborhood of the sac (29, fig. 12), instead of with the lateral pharyngeal lymphatic as is the case in the trout (fig. 10). In embryos of *Amia* measuring somewhere between 10.5 and 12.5 mm. in length and in those of *Lepidosteus* soon after they have attained the 14 mm. stage, the subocular lymph sacs (1) then become detached from the veins (fig. 13). From this time on, with the exception of a doubtful communication in

a 12.5 mm. embryo of *Amia* between the subocular sac and the lateral pharyngeal lymphatic, the subocular lymph sac, as in the trout, appears to remain detached from the veins and from the remainder of the lymphatic system during all subsequent stages of development. It is therefore seen, in both ganoids and the trout, that the subocular lymph sacs function only temporarily in the embryo as lymphatics which drain into the veins, and that the route by way of which they drain into the veins in ganoids differs from that in the trout.

Another feature of interest is that the detachment of the subocular lymph sac from the veins occurs in ganoids at a relatively much earlier stage of development, than its detachment from the lateral pharyngeal lymphatic occurs in the trout. In correlation with this early detachment of the sac in ganoids, as compared with that in the trout, the superficial facial lymphatic makes its appearance in ganoids at a relatively much earlier stage of development than is the case in the trout (compare 21 in figs. 11, 12 and 13 with fig. 10).

A comparison of the lymphatic vessels found in an advanced embryonic stage of *Amia* (fig. 13) with those found in a 17.5 mm. brook trout (fig. 11), shows that the fundamental plan of the lymphatic system in the head and pharynx is the same in each. The precardinal lymphatics (5 in fig. 13) anastomose less frequently with the lateral pharyngeal lymphatic (3) in *Amia* than is the case in the trout. In both cases the precardinal lymphatics receive the same class of tributaries, however, and drain into the veins at corresponding typical points (9, cardino-Cuvierian and 2, medial pharyngeal communications, figs. 11 and 13).

W. F. Allen ('06) has described as the 'superficial facial' lymphatic in the adult *Scorpaenichthys*, a vessel which undoubtedly corresponds to the superficial facial lymphatic mentioned above as being present in the embryos of *Amia*, *Lepidosteus* and the trout (compare 21 in figs. 11 and 13 of the trout and *Amia* with *S. Fac.* in Allen's figs. 4 and 5 of *Scorpaenichthys*). The 'profundus facial' lymphatic described by Allen in *Scorpaenichthys* may possibly correspond to the subocular lymph sac of the trout and ganoids and it is of interest to note that

Allen was unable to determine the presence of any connection between this structure and the veins in the adult fish. Allen states as follows regarding this profundus facial trunk (page 47):

"The profundus facial trunk (figs. 4 and 5, P.Fac.L.V.) could only be found in the orbit; branches were seen to enter it from the adductor mandibulae muscles, and it was traced to a point in front of the proötic foramen, where it probably passed under the jugular and entered the abdominal sinus. This point, however, could not be determined."

The 'cephalic sinus' described by Allen in *Scorpaenichthys* and the 'Kopf-Sinus' described by Jos. Hyrtl ('43) in the trout, correspond to the medial pharyngeal lymphatic (4 in figs. 13 and 11) found in the embryos of ganoids and the trout.

As the matter stands at present, considerable confusion would necessarily arise if one attempted to homologize the lymphatics as met with in the head and pharynx of adult ganoids and teleosts. Since the conditions in the adult have been found, in both cases, to be modifications of a common embryonic ground-plan, I have deemed it expedient to apply a set of terms to the lymphatics of the embryo which appear to be applicable to them, and to which the chief vessels in the head and pharynx of the adult can be uniformly referred.

What I take to be subocular lymph sacs are also met with in the embryos of selachians. In a 50 mm. embryo of *Mustelus* they were found to consist of exceedingly large semi-lunar shaped structures filled with blood, closely opposed to the medial side of the eyes, and to be in communication with the veins. A comparison of the subocular lymph sacs of fishes leads one to infer that their primitive function was to drain directly into the veins in the immediate vicinity of the sacs. It is seen that they function in this manner even at a relatively late stage of development in *Mustelus* and may possibly continue to do so in the adult; that they communicate in the same manner with the veins in ganoids during a short period of development; while in the trout and possibly in other primitive teleosts, like the trout, no direct communication is established between them and the veins, as in selachians and ganoids, in the immediate vicinity of the sacs.

I have no definite explanation to offer concerning the significance of the detachment which the subocular lymph sacs subsequently undergo from the veins in ganoids, and from the lateral pharyngeal lymphatic in the trout. One might possibly class these sacs as evanescent structures of the same general character as the posterior lymph hearts found in the embryos of certain birds. The conditions observed in ganoids, for example, are remarkably like those in birds. In the common fowl the posterior lymph hearts function only temporarily in the embryo as structures which communicate with the veins, and, although traces of these hearts can be observed in young chicks thirty-five days after hatching (Sala '00), they never enter into the formation of the lymphatic system in the adult.

Although I have not made an exhaustive study of teleosts other than the trout, I have reason to suppose that in some of the more highly specialized teleosts it is quite possible that the subocular lymph sacs, although present in the embryo, may never at any time establish a communication with the veins or with any other lymphatic. In such cases one would expect to find the superficial facial lymphatic to be developed, and to function in the capacity of draining the head region at a relatively much earlier stage of development than is the case in the ganoids and the trout.

VI. THE DEVELOPMENT OF THE SUBOCULAR LYMPH SACS IN THE TROUT

The subocular lymph sacs can be observed in the living trout embryo during the earlier as well as later stages of their development; for this reason, as well as on account of the relatively large size they attain, they lend themselves in an unequalled manner to injection experiments in the living form.

In the living trout embryo the subocular lymph sacs appear as non-pulsatile, translucent, triangular-shaped sacs filled with lymph which can easily be withdrawn from the sacs by means of a cannula. These sacs (1) lie on each side of the head, ventromedial to the eye (fig. 18), and are lined by an endothelium around which no muscular coat is formed.

By injecting into the subocular lymph sacs of the living trout embryo, as well as into other lymphatics (lymphatic of the lateral line of the trunk and lateral pharyngeal lymphatic), or, into the caudal artery or caudal vein, the following observations have been made:

1. *The subocular lymph sacs of the trout do not communicate with each other.*

2. *The subocular lymph sacs of the trout do not communicate with the veins, nor with any other lymphatic vessel during the earlier stages of their development (figs. 1 to 9, inclusive).*

In the steelhead and rainbow trout embryos for a period of from five to seven days after fertilization, and in the brook trout for a relatively longer period, each subocular lymph sac serves as a local and independent reservoir for the reception of lymph which it receives from the head region. This lymph is temporarily retained by the sac until it establishes a connection with the lateral pharyngeal lymphatic through which the sac then drains into the veins. Prior to the establishment of this connection with the lateral pharyngeal lymphatic, it has thus far proved impossible either to inject the subocular lymph sacs by way of the lateral pharyngeal lymphatic or the veins, or, to inject the lateral pharyngeal lymphatic or the veins by injecting into the subocular sacs.

Figure 18 is a photograph of a Spalteholz preparation of a twenty-day rainbow trout embryo (ventral view) on which the attempt was made to inject the lymphatic system by way of the subocular lymph sacs. The photograph shows that the subocular lymph sacs alone have received the injecta and that none of it has passed from the sacs into the lateral pharyngeal lymphatic or into the veins. Sixteen injections of this character were made of the subocular lymph sacs of the trout during the early stages of their development, and in three cases the injecta was observed to pass into the veins. On sectioning these three embryos, however, it was found that the inner wall of the sac had, in each case, been punctured by the cannula and that the injecta had extravasated into the capillaries and the veins. In other cases in which the injecta was forced into the subocular lymph

sac of a living trout embryo and the embryo kept alive for some hours, it was found that none of the injecta had passed from the sac into the lateral pharyngeal lymphatic or into the veins.

The above observations on the living trout embryo are fully borne out by a study of sections and they seem to prove conclusively that the subocular lymph sacs of the trout embryo are entirely independent of the veins and of the lateral pharyngeal lymphatic during the earliest stages of their development (figs. 1 to 9, inclusive). Injection experiments also prove that the subocular lymph sacs of the trout embryo do not increase in extent in a caudal direction.

The subocular lymph sacs of the trout therefore furnish us with a striking example of the fact that, not only do independent anlagen of the lymphatic system actually exist, but that they can also be observed and experimented upon in the living embryo. Also, in view of the circumstance that the subocular lymph sacs of the trout do not communicate with the veins during the earliest stages of their development, it is evident that they do not at first function as veins and that they therefore can not be regarded as transformed veins.

3. *The originally independent subocular lymph sacs of the trout embryo establish a temporary communication with the lateral pharyngeal lymphatic (fig. 10).*

In steelhead and rainbow trout embryos, developed at a temperature of about 10.5°C. the subocular sacs establish a direct communication with the lateral pharyngeal lymphatic on approximately the twenty-second day after fertilization. As soon as this communication has been established, the sacs can then be readily injected from the veins by way of the lateral pharyngeal lymphatic, which is the only route by means of which the injecta or blood from the veins can, at any stage of development, reach the subocular lymph sacs.

If, on account of its independent character, the presence of the subocular lymph sac happened to be unknown, it is evident by injecting into the veins or into the lateral pharyngeal lymphatic, that its presence would still remain undetected until the sac had established its connection with the lateral pharyngeal

lymphatic, and the sac might then be erroneously interpreted as an outgrowth of the latter (compare 1 in figs. 9 and 10). It is also evident if one attempted to follow the development of the lymphatics by injecting into the subocular sacs, that none of the other lymphatics already established in the embryo (lateral and medial pharyngeal and precardinal lymphatics, fig. 9), would receive the injecta until a communication had been established between the sacs and the lateral pharyngeal lymphatic; in which case one might also erroneously infer that all of the lymphatics of the head and pharynx had sprouted from the subocular sacs.

The above observations can be easily verified on living trout embryos and serve among the best examples I know of *in proof of the inadequacy of the injection method as a means of determining even the presence in the embryo of the actual anlagen of the lymphatic system*. The injection method is still consistently employed by Professor Sabin and is the one upon which her conception of the development of the lymphatics, as continuous hollow outgrowths from the veins, was originally based (Sabin '02).

4. *The subocular lymph sacs of the trout embryo subsequently become detached from the lateral pharyngeal lymphatic* (fig. 11).

As stated on a preceding page (p. 16), the subocular lymph sacs of the trout subsequently sever their connection with the lateral pharyngeal lymphatic and, from this time on, apparently neither establish any further communication with the veins, nor with any other portion of the lymphatic system. Although the independent character of the sac in the more advanced stages can be demonstrated by the injection method, the extravasations produced by forcing the injecta through the lymphaticovenous valves has proved this method to be unsatisfactory. The most conclusive proof of the independent character finally assumed by the sacs was obtained by observing the course followed by the blood in the lymphatics of the living embryo, after the latter had been subjected to a treatment of chloretone. In such embryos (see p. 10) the blood was observed to flow from the veins into the lateral pharyngeal lymphatic and thence into its anterior continuation in the head region, the superficial facial lymphatic, without entering the subocular lymph sac.

Having considered the development of the subocular lymph sacs of the trout, as far as can be observed by following the course of the injecta in the living embryo, let us now turn our attention to their study by means of sections.

The embryos were fixed in aceto-sublimate, picro-sublimate, Zenker's and Tellyesnickzky's fluids, all of which gave good results. Transverse, frontal and sagittal sections were studied, ranging in thickness from six to twenty micra. The sections were stained in borax carmine, Delafield's hematoxylin, Ehrlich's hematoxylin, Delafield's hematoxylin-orange G, Delafield's hematoxylin-eosin, and Mann's methyl blue-eosin, as recently modified by Reagan ('14). The last-mentioned stain is especially favorable for the demonstration of endothelium.

It has already been mentioned on a preceding page that the rate at which the lymphatic system develops depends largely upon the temperature of the water in which development takes place. Such being the case, it is evident, even for the same species of trout, that a particular stage of development cannot be uniformly designated as occurring upon any particular day. Even when developed under the same conditions of temperature individual variations are also of frequent occurrence; not only among different individuals of the same age, but even upon opposite sides of the same individual. When the age of an embryo is mentioned in the following pages, it must not be regarded, therefore, as uniformly related, in all cases, to the degree of development to which it is assigned.

The progressive increase in size which the subocular lymph sacs of the trout undergo during the course of development is shown in the series of reconstructions illustrated by figures 1 to 10, inclusive. These figures also show the independent character of the subocular lymph sacs (1, figs. 1 to 9, inclusive) during the early stages of development, and the connection which they secondarily establish with the lateral pharyngeal lymphatic (fig. 10):

The earliest stages in the development of the subocular lymph sacs, as observed in sections, are shown in figures 19, 20, 21, 22 and 23, which are photomicrographs of sections (10 micra in thickness) that pass through the region occupied by the anlagen

of the sacs. These five sections are all of the steelhead trout and it is unnecessary to state that the corresponding stages can also be found in brook and rainbow trout embryos. Figures 19, 20, 22 and 23 are photomicrographs of sections of injected embryos which were developed at a temperature of about 10.5°C (Series II, 1913); figure 21 is a section of another steelhead trout embryo developed at a still lower temperature and belonging to another series (Series 1912).

The very earliest indication of the anlagen of the subocular lymph sacs, as met with in the trout embryo, is shown in figure 19. These anlagen (1) appear as clear areas within a denser mesenchyme and lie lateral to the carotid arteries (22) and slightly caudal and ventro-medial to the eyes. The area occupied by these anlagen is included within the leaders in the figure. The section represented by figure 19 was cut in the frontal plane.

So much variation occurs in the rate at which the subocular lymph sac of the trout develops, that one is actually able to follow every gradation in its development, from the time of its first appearance until it has become a well-defined sac, even within a single group of embryos of exactly the same age which have developed together under similar conditions of temperature. For example, among twelve steelhead trout embryos (Series II, 1913) developed in the same tank and which were killed on the fourteenth day after fertilization, the following observations were made: In two embryos no indication of the anlagen of the subocular sacs could be observed; in one embryo the anlagen of the sacs (1) were represented as clear areas in the mesenchyme as shown in figure 19; in three embryos the anlagen of the sacs were clearly defined spaces in the mesenchyme as shown in figure 20 and, in each case, were included in one section; in one embryo both sacs were included in two sections; in one embryo the sac was included in two sections on one side of the body and in three on the other; in one embryo the sac was included in two sections on one side of the embryo and in five on the other; in one embryo the sac was included in three sections on one side of the body and in four on the other; in one embryo the sac was included in five sections on one side of the body and in four on the other,

and, in one embryo the sac was included in six sections on each side of the body (fig. 22). All of the above-mentioned sections were ten micra in thickness.

It is thus seen, within a particular group of trout embryos of exactly the same age and developed under similar conditions of temperature, that every gradation in the bilateral establishment of the subocular lymph sacs can be observed; from their very first appearance in the embryo until they have approached in size and importance a degree of development which may even be found in embryos on the fifteenth day after fertilization (fig. 23). Compare figures 22 and 23, which are photomicrographs of sections taken through the subocular lymph sacs of steelhead trout embryos, developed under similar conditions, on the fourteenth and fifteenth days, respectively, after fertilization. It is unnecessary to state that a similar set of observations could be made upon other groups of the same, or upon other groups of different species of trout embryos, which have developed under the same or under different conditions of temperature. I think there can be no question, therefore, that the clear areas (1) shown in figure 19 constitute the earliest anlagen of the subocular lymph sacs and to these we will now turn our attention.

The origin of these earliest anlagen of the subocular lymph sacs is naturally the main question at issue. Do these anlagen communicate with the veins at the time of their inception and can they be injected from the veins? Do these early anlagen at first possess what one might regard as an endothelium? Are these early anlagen of the subocular lymph sacs connected with the endothelium of the veins by solid cords of cells which might indicate that they are outgrowths from the veins?

Do the anlagen of the subocular lymph sacs communicate with the veins at the time of their inception and can they be injected from the veins?

As the result of numerous observations made on all three species of trout embryos and without exceptions, it has been my experience that the study of sections completely corroborates all of the above-mentioned observations made on the living trout embryo. Barring self-evident extravasations, I have been unable to inject

the subocular lymph sacs of the trout embryo from the veins prior to a relatively late stage of development, when a connection is then secondarily established between the sacs and the lateral pharyngeal lymphatic. It can be definitely stated, therefore, that at the time of their inception and during the early stages of their development, the subocular lymph sacs of the trout embryo do not communicate with the veins. Since the anlagen of the subocular lymph sacs cannot be injected from the veins, it is evident that the injection method neither reveals their presence in the living embryo, nor does it throw any light upon their actual mode of origin.

Do the anlagen of the subocular lymph sacs of the trout embryo at first possess an endothelium?

As shown in figures 19 and 20, the earliest anlagen of the subocular lymph sacs (1) appear as clear areas in the mesenchyme which are bounded on all sides by more closely arranged mesenchymal cells. Figures 1 and 2 are reconstructions of the left and right sides, respectively, of the embryo from which figure 20 is taken, and they show the position and relative size of the anlagen of the subocular lymph sacs (1) at about the time of their first appearance in the embryo. The anlage of the sac shown on the left side of figures 19 and 20 is more clearly defined in section than that on the right side. An examination of these clear areas (1) under high magnification shows that they have not been injected and that the clear appearance they present is due to the presence of a loosely arranged mesenchyme, between the strands of which intercellular lymph spaces are present. As these intercellular lymph spaces gradually increase in size, the anlagen of the subocular lymph sacs often present a multilocular appearance (fig. 21), due to the presence of mesenchymal strands or bridges which extend between their walls and divide the anlage of the sac into a number of compartments. These bridges rapidly break down, however, so that a single-chambered sac finally results (fig. 22). The presence of nuclei within the anlagen of the subocular sacs (left side, figs. 19 and 20), is a feature quite characteristic of developing lymph vesicles found elsewhere in the embryo. Such nuclei often appear as isolated bodies in an other-

wise clear lymph space and, as is the case of the subocular sacs, are undoubtedly related to the mesenchymal strands which formerly bridged the space.

I have been unable to determine the presence of a definite endothelium in the earliest anlagen of the subocular lymph sacs. The cells which form their walls cannot be distinguished from those of the surrounding mesenchyme. As soon as the subocular lymph sacs assume the character of definite collecting centers for lymph, the cells which form their walls begin to arrange themselves in the form of a definite endothelium. Such a condition signifies that certain cells of the embryo are undergoing a differentiation to form the definite lymphatic endothelium, otherwise it is difficult to see why transitional stages should be found.

Exactly the same transitional stages from mesenchyme to endothelium can be observed in connection with the establishment of the definite endothelium of the veins. At an early stage of development the cells which form the walls of venous channels cannot be distinguished from those of the surrounding mesenchyme and it is only later that they become spindle-shaped and assume the typical endothelial form.

Are the early anlagen of the subocular lymph sacs connected with the endothelium of the veins by solid cords of cells which might indicate that they are outgrowths from the veins?

I have also been unable to determine the presence of solid cords of cells which might extend between the endothelium of the veins and the anlagen of the subocular lymph sacs. The only veins with which such solid cords of cells could possibly be connected are the precardinal (jugular) and the infraorbital veins. The precardinal veins (6, figs. 19 and 20) lie dorsally and widely separated from the sacs. The infraorbital veins (23, figs. 1 and 2) which are tributaries of the precardinal, lie at first anterior to the anlagen of the subocular sacs, and in the embryos from which figures 19 and 20 are taken, are situated about sixty and seventy micra, respectively, anterior to these anlagen (compare 1 and 23 in figs. 1 and 2). Figure 25 is a photomicrograph of a section taken through a fourteen-day steelhead trout embryo at the level

of the infraorbital vein (23), in which region it is seen that the subocular sac has not yet made its appearance. The embryo from which figure 25 was taken is represented in reconstructions by figures 1 and 2.

In view of the observations thus far made on the living embryo and by means of sections, it may be stated that: (1) The development of the anlagen of the subocular lymph sacs of the trout is initiated soon after a definite haemal circulation has been established. The lymph present in the intercellular tissue spaces of the head region is then directed toward these anlagen which are uniformly situated at definite focal points within an otherwise densely arranged mesenchyme; (2) the gradual increase in the amount of lymph received by the subocular lymph sacs during the stage of their independence, results in the application of a constant and continuous pressure to the mesenchymal cells forming their walls, which in itself must be a positive factor in causing these cells to flatten out and gradually to assume an endothelial form. In fact, the recent experimental investigations of W. C. Clarke ('14) appear to bear out this view in a most decisive manner. Clarke's observations are as follows:

Following a purely physical injury which destroys the free surface cells of the peritoneum, pleura, or the lining cells of blood vessels, two possibilities exist as to how regeneration of the damaged zone proceeds. (1) Cells grow from the periphery of the given denuded area, taking origin from the adjacent, previously existing and intact flat surface cells; (2) the exposed deep connective tissue cells making up the floor of the injured area proliferate, change in form, becoming flattened.

These experiments were undertaken in reference to the latter possibility in the regeneration of surface cells to learn what happens as regards connective tissue cells in contact with a smooth surface, whether solid or fluid; in other words to learn what change in form takes place in the investing connective tissue cells in contact with the surface of a non-irritating body, placed for a time in the subcutaneous tissue of a living animal.

The sections showed that the cells in contact with the surface of the foreign bodies were changed in form in all of the specimens into large, flat cells placed edge to edge resulting in a definite sheet. The silver salt demonstrated a mosaic of black silvered lines marking the cell outlines of the lining of surface cells.

The fact demonstrated in the above experiments that connective tissue-cells are changed in form by physical agents into flat, closely disposed cells, the outline of which may be defined by silver salts, makes tenable the conclusion that the exposed connective tissue cells, exposed through sacrifice of surface mesothelial cells of pleura, peritoneum, or pericardium or of the lining endothelial cells of vessels, may become flattened by pressure or friction or both, resulting in regeneration of the surface cells.

Up to this point we have considered only the earliest stages in the development of the subocular lymph sacs and will now turn our attention to the later stages.

Soon after their first appearance as definite single-chambered structures, the subocular lymph sacs of the trout gradually increase in size and extent, and by the time a connection is about to be established between them and the lateral pharyngeal lymphatic, they have become relatively huge structures (fig. 42).

As shown by injection experiments and by reconstructions (figs. 1 to 10, inclusive), the subocular lymph sacs do not increase in extent in a caudal direction. The base or caudal end of the sac remains practically stationary during all stages of development and lies just in front of the hyoidean artery (15).⁴ The extension of the subocular sacs takes place in an anterior direction and can be followed through the different stages of development. During the course of their forward extension the subocular sacs gradually approach and finally come into close contact with the infraorbital veins, and it might be reasonably expected that a temporary communication, as occurs in ganoids, would be established between the independently formed sacs and the veins. Such, however, has not been found to be the case. In no instance has a communication been observed between the subocular lymph sacs of the trout and the infraorbital veins, in sections either of injected or uninjected embryos. Even if a communication were established it would not be a primary one

⁴ In the trout (fig. 10) and in *Amia* (fig. 12) the first or most anterior pair of efferent aortic arches (15) subsequently become detached from the roots of the dorsal aorta and then function as the efferent (ophthalmic) arteries of the pseudobranch (15', figs. 11 and 13). For a detailed description of the pseudobranchial arteries in adult teleosts see Silvester's paper ('04) The blood vascular system of the tile-fish (*Lopholatilus chamaeleonticeps*).

since, as already demonstrated by figures 1, 2 and 25, the subocular lymph sacs develop at first remote from the infraorbital veins (23) and only subsequently come into contact with them in the course of their forward extension. Figure 24 is a section taken through the subocular lymph sacs of a sixteen-day rainbow trout embryo in the region where the sacs (1) and the infraorbital veins (23) are in close contact with each other (compare figures 24 and 25). Although the infraorbital veins in the sixteen-day trout embryo have been injected to their fullest extent, it is seen that the subocular lymph sacs have not received any of the injecta. This has been found invariably to be the case in all trout embryos which have been injected. When blood corpuscles are met with in the subocular lymph sacs, in stages where the latter are still independent, their presence must therefore be attributed to other causes than to a connection between these sacs and the veins. Although never abundant, such corpuscles are, however, sometimes met with and, if they have not been extravasated into the sacs from the veins, or floated over into them in the balsam, they must have been derived from the walls of the sacs, or taken up from the adjacent mesenchyme.

The method by which the caliber of the subocular lymph sac increases in size and by which the original anlage of the sac extends in an anterior direction, are questions of considerable interest and importance.

Soon after the subocular lymph sac of the trout has been established in the form of a single-chambered structure, it rapidly increases in caliber. This appears to take place by division of the cells which form its walls and not by the further addition to it of mesenchymal spaces. In other words, the increase in caliber or expansion of the sac along its transverse and dorso-ventral diameters, results from a growth of the walls of the sac and, in this respect, resembles the embryonic aorta which, after it has once been laid down, gradually increases in caliber in this same manner with the growth of the embryo.

The extension of the subocular sac in an anterior direction does not appear to take place, however, in exactly the same manner. Sections taken through the anterior end of the sub-

ocular sac of the trout often present a multilocular appearance and, in this respect, resemble the conditions found in its original anlage (fig. 21). Figure 26 represents such a section taken through the anterior end of the left subocular lymph sac of a twenty-two-day rainbow trout embryo. The subocular sac is seen to be divided into three compartments by two strands of mesenchymal tissue and faint traces of strands were also observed in the sac of the opposite side when examined under high magnification. Compare figure 26 with figure 21, which is a section taken through the subocular sac soon after its first appearance in the embryo. As such compartments are not met elsewhere in the subocular sac, their presence at the cranial end of the sac in the twenty-two-day trout embryo is undoubtedly indicative of a primary and transitional condition, in which this portion of the sac is still incomplete and is still in the process of formation. Such a condition cannot be accounted for on the ground that a single-chambered anlage of the sac (1), as shown in figure 23, has gradually expanded forward. It is therefore evident that the forward extension of the subocular lymph sac takes place, not only by a growth of its walls which keeps pace with the growth of the embryo but also, like the earliest anlage of the sac (fig. 21), by the addition and confluence of independent anlagen.

VII. THE DEVELOPMENT OF THE LATERAL PHARYNGEAL LYMPHATIC IN THE TROUT

It is by means of the anlagen of the lateral pharyngeal lymphatic that a communication is first established in the regions of the head and pharynx between the lymphatics and the veins. The earliest anlagen of the lateral pharyngeal lymphatic to make their appearance in the trout embryo may establish communications with the precardinal (jugular) vein near the caudal end of the otocyst, at the cardino-Cuvierian junction, and at almost any point between the two. These points of communication may be retained in the later stages and, as far as determined, constitute the only points of communication which the

anlagen of the lateral pharyngeal lymphatic ever establish with the precardinal vein.

If the lateral pharyngeal lymphatic sprouts centrifugally, continuously and uninterruptedly from the endothelium of the veins, the supporters of the 'centrifugal' theory would necessarily maintain that it should do so only from the primary and typical points at which it can be observed to connect with the veins. Such being the case, they would also maintain that that portion of the lateral pharyngeal lymphatic which lies anterior to the points at which communications are established with the veins (between 13 and 1 in fig. 10), should be formed as the result of the forward extension of the more caudally situated anlagen of the lateral pharyngeal lymphatic which do connect with the veins. Also, in accordance with the 'tests' they have made, Sabin ('11) and E. R. Clark ('11 a) would necessarily agree that this anterior portion of the lateral pharyngeal lymphatic should always remain in continuity with the veins and that it should be potentially capable of being injected from the veins.

Let us see to what extent these conditions are fulfilled as shown by a study of injected trout embryos. We will first consider the development of that portion of the lateral pharyngeal lymphatic which lies anterior to the points at which primary communications are established between it and the veins; and second, that portion of the vessel which arises contiguous to and which communicates with the precardinal vein.

1. The development of that portion of the lateral pharyngeal lymphatic which lies anterior to the points at which primary communications are established between it and the precardinal vein

This portion of the lateral pharyngeal lymphatic, as stated above, extends between the otic communication and the subocular lymph sac (between 13 and 1 in fig. 10), and develops slightly later than the more caudally situated portion of the vessel which establishes typical communications with the precardinal vein. A study of injected trout embryos shows that this anterior portion of the lateral pharyngeal lymphatic makes

its first appearance in the form of a series of independent and discontinuous vesicles, which can neither be injected from the veins at points contiguous to them, from the subocular lymph sacs, nor from the more caudally situated anlagen of the lateral pharyngeal lymphatic which can be injected from the veins. These anterior independent anlagen subsequently become connected with one another and with the more caudally situated anlagen of the lateral pharyngeal lymphatic, to form a continuous vessel which can then be injected from the veins along its entire extent, and which finally establishes a communication with the subocular lymph sac. After this confluence is completed we meet with a condition similar to that shown in figure 10.

The progressive appearance of the independent anlagen of this anterior portion of the lateral pharyngeal lymphatic is well illustrated by a series of reconstructions which were made of injected embryos of the steelhead and rainbow trout.

In a sixteen-day steelhead trout (fig. 5) a single independent anlage (3') has alone made its appearance on the right side of the embryo (left side of figure) which lies about opposite the first efferent aortic arch (16). In a seventeen-day steelhead trout (fig. 6) similar corresponding, and other independent anlagen (3') have made their appearance on both sides of the embryo, and the position occupied by them now clearly indicates the course subsequently followed by the anterior portion of the lateral pharyngeal lymphatic. From this point on, if we follow the further development of these anterior independent anlagen (3') in the rainbow trout, we find on the twentieth (fig. 8) and twenty-first days (fig. 9) that they have also made their appearance on both sides of the embryos, and that on the twenty-first day (fig. 9) they have reached a stage of development which immediately precedes that in which they have become confluent to form a continuous vessel (3, fig. 10).

The above-mentioned reconstructions were especially chosen from among others in order to illustrate the principle that the anterior portion of the lateral pharyngeal lymphatic is formed through the concrescence of independent and discontinuous lymph vesicles. They by no means illustrate completely, how-

ever, the marked variations which occur in the rate at which these anterior independent vesicles may develop, and may become concrescent with one another, not only among different embryos of the same age, but even upon opposite sides of the same embryo. Such variations are of frequent occurrence and an example is shown in figure 5, in which it is seen that a single independent anlage (3') of the anterior portion of the lateral pharyngeal lymphatic has made its appearance only upon one side of the embryo. This question will be dealt with further on in connection with another topic (Section XI, page 50).

I will not attempt to illustrate all of the independent anlagen of the anterior portion of the lateral pharyngeal lymphatic which are shown in the figures of the reconstructions. The examination of a few typical sections taken from different embryos, at approximately corresponding levels, will suffice to show that these anlagen make their appearance in a uniform manner in a graded series of embryos, and that they therefore cannot be regarded as artifacts.

The earliest of these anlagen to make their appearance arise about opposite the origin of the first efferent aortic arches (16). An examination of the reconstructions shows such anlagen (3') to be present on the right side of the sixteen-day steelhead (fig. 5, left side) and on both sides of the embryo in all of the subsequent stages, as illustrated by figures 6 to 9, inclusive.

Transverse sections of a sixteen (fig. 27) and seventeen-day steelhead (fig. 28) and of a twenty-day rainbow trout (fig. 29) taken near the dorsal end of the first efferent aortic arches (16), pass through these earliest independent anlagen (3'). When compared with figure 30, which is a section of a twenty-two-day rainbow trout taken at a corresponding level, it is seen that the continuous lateral pharyngeal lymphatic (3) in the twenty-two-day embryo occupies the same relative position in the walls of the pharynx as its independent anlagen of earlier stages.

Transverse sections taken through a seventeen-day steelhead (fig. 31) and a twenty-day rainbow trout (fig. 32) in the region of the hyoidean arteries (15), also pass through independent anlagen (3') which correspond in their position to that occupied

by the continuous lateral pharyngeal lymphatic (3) in the twenty-two-day rainbow trout (fig. 33). The levels at which the above-mentioned figures (figs. 27 to 33, inclusive) are taken are indicated in figures 5, 6, 8 and 10.

In view of these observations, which could be multiplied indefinitely, I think there can be no doubt concerning the lymphatic character of these independent lymph vesicles and that they cannot be regarded as artifacts.

Injection experiments have shown that these independent anlagen of the anterior portion of the lateral pharyngeal lymphatic do not communicate with the precardinal vein opposite to the points at which they lie. They have also shown that these anlagen do not communicate with the subocular lymph sac, nor with the more caudally situated anlagen of the lateral pharyngeal lymphatic which do communicate with the veins. It is evident that the results obtained by the injection method do not in the least degree support the view that these independent anlagen have been derived from the veins. It is also evident that they could not be observed in following the course pursued by the injecta in the living embryo, nor in injected embryos prepared by the Spalteholz method. Since these independent lymph vesicles *are not hollow outgrowths from the veins*, there can therefore be but two possibilities regarding their origin: They must either develop *in situ* in the mesenchyme independently of the veins, or, as described by Fedorowicz ('13) and more recently by Kampmeier ('15) for the lymphatics of amphibia, be formed as the result of a secondary vacuolization within a solid cord of cells which has grown out from some preexisting endothelium. In either case the contention so often made by Huntington and the writer would still be maintained, that the lymphatics are formed by a concrescence of independent and discontinuous vascular spaces which cannot be injected from the veins. Proof that these independent anlagen are established in relation to some preexisting endothelium lies in the determination of the presence of an actual continuity between them and some definite endothelial structure. Such a continuity I have thus far been unable to find, either with the endothelium of the subocular lymph sacs,

with that of the precardinal vein at points opposite to them, or, with that of any of the more caudally situated anlagen of the lateral pharyngeal lymphatic which do connect with the veins.

As far as my own observations are concerned, the independent anlagen of the anterior portion of the lateral pharyngeal lymphatic first appear as small lymph vesicles or lymph sacs which lie often remote from the veins in an otherwise densely arranged and apparently undifferentiated mesenchyme. In later stages, however, the mesenchymal cells which bound these vesicles begin to arrange themselves in a more regular manner and to assume an endothelial form. If it would not lead to confusion, one might speak of these independent anlagen as lymph vesicles before and as lymph sacs after a definite endothelium has been formed. As far as I can determine by the aid of injections and by a study of sections, the independent anlagen of the anterior portion of the lateral pharyngeal lymphatic (between 13 and 1 in figure 10) arise *in situ* in the mesenchyme independently of any preexisting endothelium, and their mode of development appears to be a replica of that which has been described above for the subocular lymph sacs.

2. *The development of that portion of the lateral pharyngeal lymphatic which lies opposite to the points at which primary communications are established between it and the precardinal (jugular) vein*

We now pass to a consideration of those anlagen of the lateral pharyngeal lymphatic which arise contiguous to the precardinal vein and contiguous to the points at which we know the lymphatics may establish direct and typical communications with the veins. As stated above, these anlagen are confined to a district which lies between the caudal end of the otocyst and the cardino-Cuvierian junction, and we have seen that much variation also exists as regards the location and number of the communications which may be established within this district. The multiplicity of communications which these anlagen may effect with the veins, is apparently analogous to the somewhat similar

condition presented by the early anlagen of the mammalian jugular lymph sacs. It is therefore evident that the development of these anlagen should, in all probability, follow the same genetic plan as those of the mammalian jugular lymph sac.

The anlagen of the lateral pharyngeal lymphatic first to make their appearance in the embryo arise in the cardino-Cuvierian district. These anlagen (3''') are shown in figures 1 and 2, which represent reconstructions of the left and right sides, respectively, of a fourteen-day steelhead trout embryo. On the left side of the embryo (fig. 1) a single anlage (cardino-Cuvierian lymph sac, 3''') is present and lies about opposite the cardino-Cuvierian junction. On the right side of the embryo (fig. 2), however, two anlagen (3''') are met with in the cardino-Cuvierian district, one of which lies opposite the cardino-Cuvierian junction and the other slightly anterior to it.

These anlagen appear as clear areas which are situated in an otherwise densely arranged mesenchyme. Figure 34 represents a transverse section which passes through the anlage on the left side (3''', fig. 1), and through the more anterior of the two anlagen (3''') present on the right side of the fourteen-day steelhead trout embryo (3''', fig. 2). The anlage on the left side of the embryo (3''', fig. 1) is found in three sections (10 micra each) and that on the right (fig. 2) in one. On the left side of the embryo (right side of figure 34) the anlage (3''') is connected with the precardinal vein (6) by what appears to be a very narrow extension either of the vein or of the sac, and traces of the injecta can be observed in the walls of the cardino-Cuvierian lymph sac. On the right side of the embryo (left side of fig. 34) the precardinal vein sends out a blind process in the direction of the sac but does not communicate or connect with it. The more posterior of the two anlagen present on the right side of the embryo (3''', figs. 2 and 35) lies free in the mesenchyme, and no observable connection or communication exists between it and the precardinal vein. The conditions observed in this fourteen-day embryo would indicate that the cardino-Cuvierian lymph sacs of the trout are not invariably connected with the veins at the time of their inception. This is a feature of primary

significance, as it is by means of these sacs that permanent communications are subsequently established between the lymphatics and the veins in the cardino-Cuvierian district. The presence, therefore, of a connection between these sacs and the veins, as met with in certain stages, does not necessarily indicate that the cardino-Cuvierian lymph sacs have been derived from the veins, since their connection with the veins may have been formed secondarily; either through a growth or extension of the endothelium of the veins, or by a further *in situ* differentiation into endothelium of the mesenchyme that intervenes between them.

What the subsequent fate of these individual lymph sacs might be, can best be surmised by observing the conditions found in a consecutive series of embryos. An examination of such a series shows considerable variation as regards the number and location of the communications which may be established between the anlagen of the lateral pharyngeal lymphatic and the veins in the cardino-Cuvierian district. These lymph sacs (3''') may establish a communication with the veins some distance anterior to the cardino-Cuvierian junction (9, figs. 3 and 4); they may communicate with the veins at two points, one anterior and the other posterior to the cardino-Cuvierian junction (9, fig. 5, right side of embryo); they may communicate with the veins at the cardino-Cuvierian junction (9, fig. 6, both sides of embryo); they may communicate with the veins at two points at the cardino-Cuvierian junction (9, fig. 7, left side of embryo and 9, fig. 10, both sides); or, they may communicate with the veins at three points, one at the cardino-Cuvierian junction and at two points anterior to the latter (9, fig. 9, left side of embryo). An examination of the reconstructions of the later stages also shows that lymph sacs are met with in the cardino-Cuvierian district which do not communicate with the veins. Two of these independent sacs (3''') are shown in the reconstruction of a fifteen-day steelhead (fig. 3), and one on the right side of the twenty-day rainbow trout (fig. 8). In neither case have these independent sacs received the injecta, nor can any connection be observed between them and the veins.

Coincident with or soon after the appearance of the cardino-Cuvierian lymph sacs, other lymph sacs make their appearance contiguous to the precardinal vein in the neighborhood of the caudal end of the otocyst. The otic lymph sacs (3''), as these may be termed, are shown in figures 3 and 4 which are reconstructions of the left and right sides, respectively, of a fifteen-day steelhead trout embryo. These lymph sacs, like those in the cardino-Cuvierian district, may also establish a communication with the precardinal vein. On the right side of the fifteen-day steelhead trout embryo (fig. 4) the cranial end of the otic lymph sac (3'') lies fairly close to the precardinal vein (6) and, although the vein was fully injected, as shown in a section taken through the sac (3'', fig. 36) the sac is not connected with the vein (6), nor has the injecta entered the sac. On the left side of the same embryo the otic sac (3'', fig. 3) is also independent of the vein (6) but in this case an extension from the vein runs out in the direction of the sac.

As in the case of the lymph sacs in the cardino-Cuvierian district, an examination of the reconstructions of the later stages shows that variations also occur as regards the relations of the otic lymph sacs to the veins. In the sixteen-day steelhead trout embryo (fig. 5) the otic lymph sacs (3'') communicate freely with the precardinal veins on both sides of the embryo, and the relations which the sac of the right side bears to the vein resembles a condition previously described by Huntington and McClure ('10, page 203) as 'fenestration.' In the seventeen-day steelhead trout (fig. 6) and in the nineteen (fig. 7) and twenty-day (fig. 8) rainbow trout no communication exists between the anlagen of the lateral pharyngeal lymphatic and the veins in the neighborhood of the caudal end of the otocyst. In the twenty-one-day rainbow trout (fig. 9) an otic communication (13) is present on the left side of the embryo, and in the twenty-two-day rainbow trout (fig. 10), in which the cardino-Cuvierian and otic lymph sacs have become confluent to form a continuous channel, the otic communication (13) is present only on the right side.

The above observations, as illustrated by sections of injected embryos and by reconstructions, fairly represent the average conditions presented by all of the anlagen of the lateral pharyngeal lymphatic at a time before, during, and after their confluence to form a continuous vessel by means of which the subocular lymph sac drains into the veins. We have already considered in detail the development of the more anterior anlagen of the lateral pharyngeal lymphatic, and it now remains for us to consider more closely the relations which the otic and cardino-Cuvierian lymph sacs bear to the veins.

From what has been said concerning the variable relations which the otic and cardino-Cuvierian lymph sacs bear to the veins, it is evident that direct and positive proof is wanting either in favor of or against their being of venous origin; this being so for the reason that, amongst the earliest stages examined, some of these sacs were found to be independent of the veins while others were connected with the veins at the typical points of lymphatico-venous entry. In view of the circumstance that in certain instances these sacs were found to connect with the veins one might infer, in the case of those sacs which did not connect with the veins, either that they had been derived from the veins and had secondarily severed their connection with them, or, that they had developed *in situ* in the mesenchyme and had not yet established a connection with the veins at the typical points of lymphatico-venous communication. In order to prove that the otic and cardino-Cuvierian lymph sacs have been derived from the veins, it would be necessary to show that a primary continuity invariably exists between the cells which form the walls of these sacs and the endothelium of the veins. This, we have seen, has not been found to be the case.

It has already been stated that a definite endothelium which can be distinguished from mesenchyme is wanting in the subocular lymph sacs and in the independent anlagen of the more anterior portion of the lateral pharyngeal lymphatic, at the time of their inception. I may also state that what one might regard as a definite endothelium, similar to that of the veins, is also wanting

in the otic and cardino-Cuvierian lymph sacs at a corresponding stage. I therefore believe that the balance of evidence favors the view that, like the subocular lymph sac and the independent anlagen of the more anterior portion of the lateral pharyngeal lymphatic, the lymph sacs in the otic and cardino-Cuvierian districts develop *in situ* in the mesenchyme and that, while certain of these sacs may secondarily establish venous connections, others may enter directly into the formation of the lateral pharyngeal lymphatic without previously joining the veins.

Felix ('97) has described the development of the aorta and cardinal veins in the trout and salmon and finds that these vessels not only arise *in situ* but that their lumina are formed by a confluence of vascular spaces which make their appearance in a discontinuous manner in a solid cord of cells. The recent experimental work of Stockard ('15) on *Fundulus* has verified these results in all essential details. If we compare the development of the lateral pharyngeal lymphatic with that of the aorta and cardinal veins, we find that the lateral pharyngeal lymphatic, like the aorta and the cardinal veins, presents a temporary ontogenetic condition in which a continuous channel is invariably preceded by a series of discontinuous vascular spaces. This is an incontrovertible fact which can be definitely established by the aid of the injection method and by a study of sections. Such a similarity certainly signifies a common genetic plan of development for all of these independent vascular spaces, and for the endothelium which forms their walls, rather than that the plan of development for the lymphatics differs from that of the arteries and veins. This question will be more fully considered in connection with another topic (Section XIII, p. 57).

VIII. THE DEVELOPMENT OF THE SUPERFICIAL FACIAL LYMPHATIC IN THE TROUT

It has already been stated that the subocular lymph sac of the trout embryo subsequently severs its connection with the lateral pharyngeal lymphatic (page 23) and that the latter is then continued into the head region as a vessel which has been designated as the superficial facial lymphatic. This vessel (21) is

shown in the reconstruction of the 17.5 mm. brook trout (fig. 11) and in section in an 18.25 mm. rainbow trout (21, fig. 17).

It has proved a more difficult matter to follow the development of the superficial facial lymphatic than that of any of the other main lymphatic vessels found in the trout. One reason for this difficulty lies in the circumstance that the superficial facial lymphatic develops in an extremely loose mesenchyme the cells of which, in the earlier stages of development, cannot be distinguished from those that line the subocular lymph sac, or, from those that form the walls of the superficial facial lymphatic. Even in the later stages when the superficial facial lymphatic can be easily injected and its presence positively established, it is often quite impossible to distinguish any difference between its endothelial wall and the contiguous mesenchymal cells. It is therefore only with difficulty, and in a most favorable series of sections, that the earliest anlagen of the superficial facial lymphatic can even be identified at all.

The earliest anlage of the superficial facial lymphatic that could be identified by the writer may be described as it appeared in a rainbow trout embryo on the twenty-third day after fertilization. The anlage could be determined definitely in this particular embryo only upon the left side of the body. On the right side of the embryo, if present, the anlage was indistinguishable in section from a mesenchymal cleft. On the left side of the embryo the anlage of the lateral pharyngeal lymphatic consisted, in part, of a short vessel which communicated with the lateral pharyngeal lymphatic near a point where the latter opened into the subocular lymph sac; and, in part, of an entirely independent and separate anlage. The former could be followed anteriorly through fourteen sections and is shown in section in figure 37, at the point where it (21) connects with the lateral pharyngeal lymphatic and the subocular lymph sac (1), and in figure 38 eleven sections anterior to the latter.

In principle, the plan of development of the superficial facial lymphatic is similar to that of other main lymphatic channels. It develops forward into the head region, by the gradual addition to the anterior end of the lateral pharyngeal lymphatic, of

mesenchymal strands, between which clefts or vesicles—the future lumen of the vessel—make their appearance. If one were to follow its development solely by means of the injection method, the superficial facial lymphatic would present the appearance of gradually growing continuously forward from the anterior end of the lateral pharyngeal lymphatic. As a matter of fact, however, such an appearance would only be simulated, since its forward extension, and the extent to which it can be injected, are directly dependent upon the extent to which its independent anlagen have become conrescent with the lateral pharyngeal lymphatic.

IX. THE DEVELOPMENT OF THE PRECARDINAL (JUGULAR) LYMPHATICS IN THE TROUT

In advanced embryonic stages of the trout (fig. 11) and of *Amia* (fig. 13) the precardinal lymphatics (5) form an extensive system of vessels which communicate indirectly with the veins by means of the lateral and medial pharyngeal lymphatics.

The development of the precardinal lymphatics parallels that of the lateral pharyngeal and superficial facial lymphatics just described. Discontinuous lymph vesicles make their appearance along the course of the precardinal veins. These discontinuous anlagen subsequently become connected with one another and with the lateral pharyngeal lymphatic, at about the level of the otic lymph sac, to form a system of vessels which can then be injected from the veins. Prior to the connection of these anlagen with one another and with the lateral pharyngeal lymphatic, I have been unable to inject them from the veins without producing extravasations.

The anlagen of the precardinal lymphatics first to make their appearance arise at about the level of the otic lymph sacs. These anlagen (5'') are shown in figures 4 to 8, inclusive. Sooner or later (figs. 5, 9 and 10), a communication (7) is invariably established between them and the lateral pharyngeal lymphatic in the region of the otic communication (13). In a fifteen-day steel-head (fig. 4) a single anlage (5'') has made its appearance on the

right side of the embryo which may possibly communicate with the otic lymph sac (3'') through an exceedingly narrow intercellular connection. This anlage (5''), as shown in section (fig. 36), lies closely in contact with the precardinal vein (6) and, as the otic lymph sac (3'') does not communicate with the vein, neither the otic lymph sac nor this anlage of the precardinal lymphatic has received any injecta from the vein. In a sixteen-day steelhead trout (fig. 5) two of these earliest anlagen (5'') have made their appearance and they not only communicate in this embryo with the otic lymph sacs (3''), but also through the latter with the veins at the otic communication (13). From the conditions shown in the fifteen and sixteen-day steelhead trout embryos, one might infer that these earliest anlagen of the precardinal lymphatics were not formed independently of the otic lymph sacs but were extensions of the latter, and such may possibly be the case for these particular anlagen. In view of the circumstance, however, that in relatively much later stages of development (figs. 6, 7 and 8) these same anlagen (5'') may also be found to be independent of the otic lymph sacs (3''), I am lead to believe that they arise independently of the latter and secondarily connect with them. In other words, the independent character of these anlagen (5'') in figures 6, 7 and 8, probably indicates a condition in which the establishment of a connection between them and the otic lymph sacs (3'') has been temporarily delayed (see Section XI, p. 50).

We now pass to a consideration of those independent anlagen of the precardinal lymphatics which arise remote from the otic lymph sacs and remote from the typical points at which the lymphatics of the trout communicate with the veins.

In general, these anlagen of the precardinal lymphatics may be divided into two groups: One of which is situated posterior and the other anterior to the anlagen which arise contiguous to the otic lymph sacs. The position occupied by the more posterior anlagen is shown on the left side of figure 7 which is a reconstruction of a nineteen-day rainbow trout embryo. This anlage (5''') lies at about the level of the third efferent aortic arch (18) and, as far as can be determined, does not communicate with the

precardinal vein (6), with the anlage (5'') near the otic lymph sac, nor is it connected with the vein by a solid endothelial strand. In the twenty-two-day rainbow trout (fig. 10) this anlage (5''') connects with the precardinal sac (5'') which arises near the otic communication and with the former forms a continuous vessel or sac (5) which can now be injected from the veins.

The only point at which an extraintimal replacement of a degenerating vein by a developing lymphatic could occur in the trout would be in connection with the precardinal lymphatics although, as far as I have been able to determine, such a replacement does not take place. It is evident, however, if the precardinal vein (6) in figure 39 were to undergo atrophy and the precardinal lymphatic (5) to increase in size, we would then have a typical example of the extraintimal replacement of a vein by a lymphatic, as described by Huntington and the writer in connection with the development of the thoracic ducts and mesenteric lymphatics in mammals.

In addition to the posterior anlagen of the precardinal lymphatics just mentioned, additional independent anlagen arise in the region anterior to the otic lymph sacs. These anlagen (5') make their appearance on about the twenty-first day in the rainbow trout and, as shown in the reconstruction of the twenty-two-day rainbow trout embryo (fig. 10) they lie remote from the point at which they will subsequently connect with the lateral pharyngeal lymphatic (7). These anlagen (5') are also shown in section (fig. 33) in which it is seen that they arise contiguous to the precardinal vein (6).

The question concerning the method by means of which connections are established between the independent anlagen of the lateral pharyngeal lymphatic, and between the lymphatics and the veins, is of secondary consideration at present and will be fully considered further on (Section XIII, p. 57). The main question at issue is whether or not such independent endothelial-lined anlagen actually exist. If they do exist, the contention that lymphatic endothelium is specific, in the sense that it is invariably derived from that of the veins, can no longer be

maintained and the theory that lymphatic endothelium, in general, is of venous origin would then necessarily fall completely to the ground.

X. THE DEVELOPMENT OF THE MEDIAL PHARYNGEAL LYMPHATIC

The earliest continuous vessel in the trout embryo which conveys lymph from the head and pharynx to the venous circulation is the lateral pharyngeal lymphatic, which may communicate with the veins in the cardino-Cuvierian and otic districts of communication. Although the otic communication is wanting, this stage is represented on the left side of the twenty-two-day rainbow trout embryo (right side of fig. 10). This early plan of drainage soon becomes modified in the trout embryo, however, by the formation of a new lymphatic channel, the medial pharyngeal lymphatic (4, fig. 10). This vessel taps the lateral pharyngeal lymphatic secondarily and then directs the lymph from the latter to the precardinal (jugular) vein (6), with which it communicates at a point near the exit of this vein from the cranial cavity (2, medial pharyngeal communication, left side of fig 10). This modified plan of drainage has been established on the right side of the twenty-two-day rainbow trout embryo (left side of fig. 10; see also 4 in fig. 11).

From what has been stated above, it is evident that the medial pharyngeal lymphatic makes its appearance in the trout embryo at a relatively late stage of development, and that it then serves only in the capacity of conveying to the venous circulation by an additional route, lymph originally collected by an independently formed system of lymphatics (subocular lymph sac and lateral pharyngeal lymphatic).

In a series of rainbow trout embryos which were developed at a temperature of about 10.5°C, the medial pharyngeal lymphatic made its first appearance between the twentieth and twenty-first days. On the twenty-first day (4, fig. 9) it was found to be present on both sides of the embryo and to be connected with the veins at the medial pharyngeal communication (2, fig. 9).

We have already seen that the constituent vessels of which the embryonic ground-plan of the lymphatic system is composed are the same in ganoids as in the trout. It is interesting to note, however, that the order in which these vessels appear in the embryo, in the course of their ontogenetic development, is somewhat different in ganoids than in the trout.

In ganoids (fig. 12) the medial pharyngeal lymphatic develops into a large and prominent sinus (4) which drains the lateral walls of the head (21) and pharynx before a continuous lateral pharyngeal lymphatic (3'') has been formed. In the trout embryo, on the other hand, the medial pharyngeal lymphatic (4, fig. 10) is one of the last of the principal lymphatics of the pharynx to be developed, and it then serves only in a subsidiary capacity, after connecting with the lateral pharyngeal lymphatic, of aiding the lymph collected by the latter to drain into the veins. In the earlier embryonic stages of ganoids we therefore find that the medial pharyngeal lymphatic plays a more important rôle than the lateral pharyngeal lymphatic in conveying lymph to the venous circulation, while the reverse is the case in the trout.

The superficial facial lymphatic (21, fig. 12) also makes its appearance in the embryos of ganoids (*Amia* and *Lepidosteus*) before a continuous lateral pharyngeal lymphatic has been formed. In the trout embryo, on the other hand, the superficial facial lymphatic does not make its appearance until a relatively late stage of development, and until after a continuous lateral pharyngeal lymphatic has been formed (21, fig. 11; compare with fig. 10).

In the more advanced stages, in which a continuous system of lymphatics has been established, the medial pharyngeal lymphatic serves in a similar manner, in both the trout and ganoids, as a prominent pathway or sinus, through which the lateral pharyngeal and superficial facial lymphatics drain into the veins (4, figs. 11 and 13).

For the reason that the medial pharyngeal lymphatic is a structure of relatively greater importance in the embryos of ganoids than in those of the trout, the earliest stages of its de-

velopment are more easily followed in ganoids than in the trout. The earliest anlage of the medial pharyngeal lymphatic was met with in a 7.5 mm. embryo of *Amia* (6.81 mm., measured by sections) and is shown in section in figure 40. This anlage (4') was included in two sections (10 micra each) and, as shown in the figure, it neither communicates with the precardinal vein (6), nor is it connected with the latter in this, or in any other section, by a solid endothelial strand. A study of the later stages shows that this independent anlage invariably establishes a connection with the precardinal vein (4, figs. 12 and 13), and it can then be easily injected from the veins before lymphatico-venous valves are formed. The vascular system of the embryo from which figure 40 is taken was fully reconstructed in order to prove that the anlage in question was that of the medial pharyngeal lymphatic. Since this independent anlage was found to occupy identically the same relative position in this embryo as that of the medial pharyngeal sac of later stages, no possible doubt can exist regarding it.

The anlagen of the lymphatic system, which develop in close relation to the typical points at which the lymphatics establish communications with the veins, have been described by Huntington and McClure ('10) in mammals as 'venolymphatics.' The term 'venolymphatic' may therefore be similarly applied to those anlagen of the lymphatic system of fishes which develop contiguous to the typical points at which the lymphatics communicate with the veins. These anlagen include the otic, the cardino-Cuvierian and the medial pharyngeal lymph sacs in the trout; the cardino-Cuvierian, the subocular and medial pharyngeal lymph sacs in ganoids. In view of the interpretation placed by the writer upon the genesis of the lymphatics in general, the use of the term 'venolymphatic,' we shall see (Section XIII, p. 57), becomes entirely unnecessary in distinguishing these anlagen of the lymphatic system from those which develop elsewhere in the body remote from the typical points of lymphatico-venous entry. The medial pharyngeal lymphatic of *Amia* serves to illustrate the principle of lymphatic, as well as that of vascular development, in general, for the reason that, being classed as a 'veno-

lymphatic,' it can be demonstrated that its anlage first appears in the mesenchyme independently of the veins, and that a connection between it and the veins is established secondarily. It is highly improbable that homologous structures, like the subocular lymph sacs of fishes, could differ fundamentally in their mode of development. We have seen that the subocular lymph sacs of ganoids would be classed as 'venolymphatics,' on the basis of the direct connections which they establish with the veins in the immediate neighborhood of the sacs; the subocular lymph sacs of the trout, however, could not be designated as 'venolymphatics' on this basis, since they never communicate with the veins in the neighborhood of the sacs. The development of the subocular lymph sacs of fishes therefore serves as another illustration of the fact that no distinction really exists between 'venolymphatics' and other anlagen of the lymphatic system.

XI. VARIATIONS IN THE RATE AT WHICH THE LYMPHATIC SYSTEM OF THE TROUT DEVELOPS IN DIFFERENT EMBRYOS OF THE SAME AGE, AS WELL AS UPON OPPOSITE SIDES OF THE SAME EMBRYO

It has already been stated that much variation exists in the rate at which the lymphatic system develops in different individuals of the same age, as well as upon opposite sides of the same individual. This variability has been partially illustrated in the figures of the reconstructions, but is more fully borne out by the following observations which were made in following the course of the injecta in the lateral pharyngeal lymphatic of the living embryo. In order to illustrate this variability more fully, I will give the results observed in connection with a particular series of steelhead trout embryos which were developed at a temperature of about 10.5°C and injected under the binocular microscope. All injections were made through the caudal vein.

In this particular series of trout embryos the lymphatic system could not be injected until the fourteenth day after fertilization. On the fourteenth and fifteenth days after fertilization the cardino-Cuvierian and otic lymph sacs could be injected in a few

cases, although in most of the embryos injected no indications of these sacs could be observed.

On the sixteenth day the following observations were made upon sixteen (16) embryos:

In three embryos the injecta could be forced into the lateral pharyngeal lymphatic from the cardino-Cuvierian communications, as far forward as the caudal end of the otocyst, on both sides of the body; in one embryo the cardino-Cuvierian lymph sac could be injected on one side of the body; in the remaining twelve embryos no definite observations could be made concerning the presence of lymphatic anlagen, either at the cardino-Cuvierian or otic points of communication.

On the seventeenth day the following observations were made upon eleven (11) embryos:

In four embryos the lymphatics could not be injected even under pressure; in two embryos a small cardino-Cuvierian lymph sac was filled with the injecta on each side of the body; in five embryos the lateral pharyngeal lymphatic could be injected as far forward as the caudal end of the otocyst on both sides of the body and, in three of these embryos, this vessel appeared to communicate with the veins at both the cardino-Cuvierian and otic points of communication.

On the eighteenth day the following observations were made upon twenty-one (21) embryos:

In eighteen embryos the lateral pharyngeal lymphatic could be injected on both sides of the body as far forward as the caudal end of the otocyst and, in about half of the embryos, it connected with the veins at the cardino-Cuvierian and otic points of communication; in two embryos the lateral pharyngeal lymphatic could be injected on both sides of the body to about the middle of the otocyst, and in one embryo it could be injected only half way to the otocyst on one side of the body and to the caudal end of the otocyst on the other.

On the nineteenth day the following observations were made upon eighteen (18) embryos:

In four embryos the subocular lymph sacs could be injected for the first time through the lateral pharyngeal lymphatic, but

only on one side of the body, while the lateral pharyngeal lymphatic of the opposite side of the same embryo injected to the caudal end of the otocyst in one case, to the middle of the otocyst in two cases and as far forward as the eye in the other; in three embryos the lateral pharyngeal lymphatic could be injected as far forward as the eye on one side of the body, while the lateral pharyngeal lymphatic of the opposite side injected to the middle of the otocyst in one of these embryos and to the caudal end of the otocyst in the remaining two; in five embryos the lateral pharyngeal lymphatic could be injected only to the caudal end of the otocyst on both sides of the body; in one embryo the lateral pharyngeal lymphatic could be injected as far forward as the anterior end of the otocyst on both sides of the body; in one embryo the lateral pharyngeal lymphatic could be injected to the caudal end of the otocyst on one side of the body and to the middle of the otocyst on the other; in two embryos the lateral pharyngeal lymphatic could be injected to the anterior end of the otocyst on one side of the body and, only to the caudal end of the otocyst on the other; in two embryos only a small cardino-Cuvierian lymph sac could be injected on one side of the body, while the lateral pharyngeal lymphatic of the opposite side injected to the caudal end of the otocyst in one of these embryos, and to the anterior end of the otocyst in the remaining two.

On the twentieth day the following observations were made upon twelve (12) embryos:

In three embryos the subocular lymph sacs could be injected through the lateral pharyngeal lymphatic on both sides of the embryo; in two embryos the subocular lymph sac could be injected through the lateral pharyngeal lymphatic on one side of the embryo (fig. 15), while the lateral pharyngeal lymphatic of the opposite side injected up to the subocular sac but did not communicate with it; in one embryo the subocular lymph sac could be injected through the lateral pharyngeal lymphatic on one side of the body, while the lateral pharyngeal lymphatic of the opposite side injected only to the caudal end of the otocyst; in two embryos the subocular lymph sacs could be injected through the lateral pharyngeal lymphatic on one side of the body, while

the lateral pharyngeal lymphatic of the opposite side could not be injected under pressure; in two embryos the lateral pharyngeal lymphatic could be injected on both sides of the body, only as far forward as the caudal end of the otocyst; in one embryo the lateral pharyngeal lymphatic could be injected on both sides of the body as far forward as the subocular lymph sacs but did not communicate with them; in one embryo the lateral pharyngeal lymphatic could be injected only to the caudal end of the otocyst on one side of the body, and to the anterior end of the otocyst on the other.

On the twenty-first day the following observations were made upon thirteen (13) embryos:

In five embryos the subocular lymph sacs could be injected on both sides of the body through the lateral pharyngeal lymphatic; in six embryos the subocular lymph sac could be injected only on one side of the body and, in each case, the lateral pharyngeal lymphatic of the opposite side injected up to the sac but did not communicate with it; in one embryo the lateral pharyngeal lymphatic could be injected only as far forward as the caudal end of the otocyst; in one embryo the lateral pharyngeal lymphatic could be injected on both sides of the body as far forward as the middle of the otocyst.

On the twenty-second day both subocular lymph sacs could be injected through the lateral pharyngeal lymphatic in almost every case (about 90 per cent). Soon after the establishment of a connection between the subocular lymph sac and the lateral pharyngeal lymphatic, valves begin to be formed at the typical points at which the lymphatics permanently communicate with the veins. It then becomes more difficult to inject the lymphatics from the veins without causing extravasations.

I fully appreciate that the above-mentioned results apply to the conditions found in relatively few embryos and that, in all probability, the injection may not, in all cases, have been complete. In view of the fact, however, that essentially similar observations were made upon eight other separate occasions, I feel that they represent, to a very fair extent, the conditions as they actually exist.

If we analyze these observations more closely, we find that up to the eighteenth day the anlagen of the lateral pharyngeal lymphatic cannot, in all cases, be uniformly injected from the veins at the typical points of lymphatico-venous entry. From the eighteenth day on, however, with one exception on the twentieth day, no difficulty was experienced in forcing the injecta into the anlagen of the lateral pharyngeal lymphatic from the veins. The study of sections of injected embryos has shown that the inability to inject the anlagen of the lateral pharyngeal lymphatic in the earlier stages is due to the circumstance that, in some cases, they do not communicate with the veins, or, if a communication is present, it may be too narrow to permit the passage of the injecta into the anlagen from the veins.

On the eighteenth day we meet with the nearest approach to a uniformity, in which the lateral pharyngeal lymphatic has attained a stage of development which might be regarded as typical for this particular day. At this stage (in 18 of the 21 embryos injected) the lateral pharyngeal lymphatic was represented by a continuous vessel on both sides of the embryo which extended between the cardino-Cuvierian junction and the caudal end of the otocyst, *at which point, in some cases, it communicated with the veins*. This same condition was also met with to a lesser degree on the sixteenth (in 3 of the 16 embryos injected) and seventeenth days (in 5 of the 11 embryos injected) and is illustrated by figure 6, which represents a reconstruction of a seventeen-day steelhead trout embryo. This condition signifies, I believe, the attainment of a necessary preliminary stage of development in which the establishment of the lymphatic drainage system for the posterior portion of the pharynx may be regarded as complete. This drainage system for the posterior region of the pharynx is still independent, however, of that of the subocular lymph sac.

From the eighteenth to the twenty-second day no indication of a uniform condition was met with and, during this period, the further development of the lymphatic system is chiefly concerned in establishing a communication between this posterior drainage system of the pharynx and the subocular lymph sac. A study of sections of these injected trout embryos has shown

that a series of independent lymph vesicles intervene between these two primary drainage centers and that they lie in the course subsequently followed by the lateral pharyngeal lymphatic. It has also been shown that the subocular lymph sacs cannot be injected from the veins until this intervening portion of the lateral pharyngeal lymphatic is completed and until the latter has established a connection with the subocular lymph sac. Such being the case, it is evident that the pronounced variability observed on the nineteenth, twentieth and twenty-first days, must be related to the difference in the rate at which a connection has been established between the primary posterior drainage center of the pharynx, and the independent anlagen (independent lymph vesicles) of the lateral pharyngeal lymphatic which intervene between the primary posterior drainage system of the pharynx and the subocular lymph sac.

XII. DO THE PERIPHERAL LYMPHATICS FORM A CLOSED SYSTEM OF ENDOTHELIAL-LINED VESSELS SIMILAR TO THOSE OF THE HAEMAL SYSTEM?

The presence of a closed system of peripheral lymphatics is generally regarded as a necessary corollary to the theory that the lymphatics sprout centrifugally and continuously throughout the body of the embryo from the endothelium of the veins. On the other hand, it is generally inferred that the view which calls for a local origin of lymphatic endothelium from tissues other than from a preexisting venous endothelium, demands the presence of a permanent open communication between the peripheral lymphatics and the intercellular tissue spaces. I may state that the falsity of this inference becomes apparent when we consider that recent investigations have conclusively shown that the endothelium of the entire blood-vascular system of vertebrates—which is a closed system—possesses a local origin. It is therefore evident that the view which calls for a local *in situ* origin of lymphatic endothelium, and for the development of lymphatic channels through the confluence of independent lymph vesicles, does not necessarily preclude the possibility that a closed system of

peripheral lymphatics may exist and, if such is actually the case, the question can alone be decided by a direct observation of the facts.

I have hitherto paid little attention to this particular subject. I have made, however, certain observations which bear more or less directly upon this point.

In sections of the embryo one frequently meets with a condition in which the lumen of an endothelial-lined lymphatic opens directly into a mesenchymal-lined tissue space. One of the best and most convincing examples of this character was demonstrated by Huntington at a meeting of the American Association of Anatomists in 1911 and he has kindly permitted me to illustrate it here. Figure 41 is a photomicrograph of a transverse section taken through the periaortal lymph sinus of *Chelydra serpentina*. According to Huntington this lymph sinus is not connected with any vein and has developed in the mesenchyme in an area entirely free of veins. As shown clearly in the photomicrograph, this lymph sinus (25) is lined by a continuous layer of endothelium except at one end (right side of fig. 41) where the endothelium ends abruptly and the lumen of the lymph sinus opens directly into a typical mesenchymal-lined tissue space (26).

The increase in extent which this lymph sinus of *Chelydra* was still undergoing, at the time the embryo was killed, was being accomplished, I believe, as the result of an addition to it of this mesenchymal tissue space (26), and the continued addition to the latter of similar adjacent tissue spaces (32) would further increase its extent. It is evident that this lymph sinus of *Chelydra* with its communicating tissue space can in no sense be regarded as a closed endothelial-lined tube. If it should ultimately become one, this could be accomplished only as the result of a further local *in situ* differentiation into endothelium of the entire mesenchymal strand which forms the wall of the adjacent communicating tissue space. It is quite possible that in the course of the subsequent extension of this lymph sinus, by the continued addition to it of mesenchymal spaces, a stage might ultimately be reached in which the mesenchymal wall of a communicating tissue space became differentiated into endothelium

along its entire extent; in which case a closed endothelial-lined tube would result. It is also quite possible that the conditions actually observed in the embryo of *Chelydra* might persist in the adult. Whatever may ultimately be proved to be the case for the peripheral lymphatics, in general, it would seem best for the present to withhold an opinion until a positive demonstration is at hand.

XIII. GENERAL CONSIDERATIONS CONCERNING THE GENESIS
AND GROWTH OF THE LYMPHATIC SYSTEM IN ITS
RELATIONS TO THE HAEMAL SYSTEM^a

Does the endothelium of the lymphatic system arise, at any time or place, in a discontinuous manner and independently of that of the veins? As we shall see, the determination of this question constitutes a solution of the lymphatic problem.

The view that lymphatic endothelium spreads continuously and uninterruptedly throughout the body of the embryo from the endothelium of the veins, is merely an extension, and application to the endothelium of the lymphatic system, of the well-known view held by His, that the endothelium of the intra-embryonic haemal vessels grows continuously and uninterruptedly into the embryo from the yolk-sac angioblast. Such a method of origin necessarily implies that all intra-embryonic endothelium arises only from a preexisting endothelium which takes its origin in the yolk-sac, and that in the body of the embryo a discontinuity of origin never occurs.

The view opposed to the 'ingrowth' or 'angioblast' theory of His has been closely associated with the names of Rückert and Mollier ('06). This view consists in the claim that the endothelium of the intra-embryonic haemal vessels develops *in situ* in the body of the embryo, and that it is not derived from the yolk-sac angioblast.

^a Section XIII has already been published in practically its present form under the title of 'The development of the lymphatic system in the light of the more recent investigations in the field of vasculogenesis' (McClure '15 b).

Since the lymphatics merely represent a component part of a general vascular system, to which the haemal vessels also belong, the probability at least, is that, in the genesis of their endothelium, and in the establishment of a continuous system of vessels, the lymphatic and haemal vessels should follow a common genetic plan. Let us consider, in the light of the more recent investigations in the field of the vascular system, what this plan may be.

It is not necessary to review in detail the investigations of those who have hitherto maintained that the endothelium of the intra-embryonic blood-vascular system possesses a local origin. For an historical and critical account of this subject it is sufficient to refer to the investigations of Rückert and Mollier ('06), Felix ('97) and to the more recent one by Schulte ('14) on the development of the intra-embryonic blood-vascular system of mammals.

It should be clearly borne in mind that, until quite recently, the investigations which have dealt with the origin of intra-embryonic vascular endothelium have not been experimental in character, but have been based largely upon a study of fixed material in which, however, a local and discontinuous origin of blood-vascular anlagen has been observed.

Let us now see how the view that the endothelium of the intra-embryonic blood-vascular system develops *in situ*, and does not grow into the embryo from the yolk-sac, is borne out by experiment.

Two types of experiment have thus far been made to determine this question: (1) The partial separation of the embryo from the yolk-sac, or, the complete separation and isolation of a portion of the embryo from the rest of the embryo and from the yolk-sac, at a time prior to the possible invasion of the embryonic axis by the yolk-sac angioblast; (2) by observing the effects produced on the developing blood-vascular system in embryos which have been allowed to develop under the influence of anaesthetics or other chemical agents.

The experimental investigations of Hahn ('09), and Miller and McWhorter ('14) have shown, by effecting a separation on one

side between the body of the chick embryo and the yolk-sac, before vessels have appeared in the area pellucida, that blood vessels make their appearance in the body of the embryo in a typical manner on the operated side. These vessels differ from those on the unoperated side only in size and rate of development, differences which may be correlated with their reduced drainage area and the consequent diminished quantity of circulatory fluid.

These experiments of Hahn, and Miller and McWhorter have conclusively shown that the yolk-sac angioblast cannot have grown into the embryo on the operated side. In order to eliminate the possibility, however, that the vessels on the operated side may not have been formed *in situ*, but by an invasion of angioblast from the normal unoperated side, Reagan ('15) has recently performed a set of experiments in my laboratory which conclusively disprove this contention. Instead of separating only one side of the embryo from the yolk-sac, Reagan has been able to develop the heads of chick embryos, which had been completely separated from the rest of the embryo and from the yolk-sac, and in which endothelial-lined vascular channels of mesenchymal origin were invariably found to appear. As in the case of the experiments of Miller and McWhorter, the operations were performed at a time before it would have been possible for the intra-embryonic tissue to have been invaded by yolk-sac angioblast.

Gräper ('07), under the direction of C. Rabl, performed a set of experiments on chick embryos, somewhat similar to those of Hahn, and Miller and McWhorter, and, although he noted the presence of independent blood-islands in the body of the embryo, he was unable to interpret them as having been formed *in situ*.

Jacques Loeb ('12) was the first to observe the effects produced by certain chemicals (NaCN) on the developing blood vessels in fish embryos. He was able to produce a condition in which a beating heart and blood were present, but no circulation; a condition which, as stated by Schulte, can hardly be reconciled with the doctrine that the vessels of the embryo have a primitive

continuity of lumen with those of the yolk-sac, for it is inconceivable that in such circumstances, a beating heart could fail to effect a circulation.

The investigations of Stockard ('15) supplement and coincide with those of Hahn, Miller and McWhorter, Reagan, and Loeb in a most decisive manner. Stockard has shown that, not only do anesthetics arrest the development of the intra-embryonic blood vessels in the embryos of *Fundulus*, at an early ontogenetic stage, but in such a manner that no doubt can now exist that, under normal conditions, these vessels are formed *in situ* by a concrescence of independent and discontinuous anlagen, and that their endothelium is derived directly from intra-embryonic mesenchymal cells. It is interesting to note in this connection that Wenkebach ('86) had already observed in the body and yolk-sac of the living fish embryo (*Belone longirostris*), that mesenchymal cells play an important rôle in the formation of vessels and sprouts. In their general features the observations of Wenkebach have been confirmed by Raffaele ('92).

It is thus seen that experimentation bears out the observations made upon fixed and living material, that the intra-embryonic blood-vascular channels do not grow into the embryo from the yolk-sac, but are formed *in situ* by a concrescence of independent and discontinuous anlagen, whose endothelium is formed from intra-embryonic mesenchymal cells.

The vascular plexus formed in the extra-embryonic area of the vertebrate embryo, is as we know, at first represented by discontinuous, independent and circumscribed anlagen, the cells of which possess a local origin. Clefts or spaces, the future lumina of the plexus, soon make their appearance in a discontinuous manner amongst the cells of these anlagen, and it is by a concrescence of these vascular spaces that a continuous system of vascular lumina is finally formed. The cells which constitute the walls of these vascular spaces become transformed into the endothelium and, when blood-islands are present, the more centrally situated cells form the primary blood cells. It is interesting to note in this connection that McWhorter and Whipple ('12) have recently been able to demonstrate and record

photographically the concrescence of separate vascular anlagen in the area pellucida of the chick's blastoderm *in vitro*.

If we compare the development of the intra-embryonic blood-vascular channels, as determined by observation and experiment, with that of the plexus which arises on the yolk-sac, we find, in the genesis of their endothelium from mesenchyme, and in their formation by a concrescence of independent anlagen, that the intra- and extra-embryonic blood-vascular channels follow exactly the same genetic plan.

If one attempted to follow the development of these intra- or extra-embryonic blood-vascular channels by means of injections, it is evident that this method would reveal only the extent to which a continuous system of injectible lumina had been established at the time the injections were made. It would fail completely to reveal the facts which have been definitely determined by experiment, that the injectible lumina had been previously formed by a concrescence of independent and uninjectible vascular spaces, and that the endothelium which forms the walls of these lumina had been formed *in situ*, not from a preexisting endothelium, but from mesenchymal cells.

Since we now know that the intra-embryonic blood vessels, like those in the yolk-sac, are formed by a concrescence of independent anlagen, and that their endothelium is formed *in situ* from mesenchymal cells, the question naturally confronts us as to the method by means of which these independent anlagen become connected with one another to form a system of vessels with continuous lumina, that extend throughout the body of the embryo.

There appear to be only three possible methods by means of which such connections could take place: (1) Either by means of a proliferation or migration of the cells of which the original independent anlagen are composed; (2) by a further local *in situ* differentiation into endothelium of the embryonic cells which intervene between the independent anlagen; or (3) by a combination of these two methods.

We all recognize the fact that endothelium, like other tissues of the body, is capable of growth after it has once been formed.

In no other manner could we account for the increase in size which blood vessels undergo in the embryo after they have attained their adult structure and form. It is also possible for anastomoses to be formed between different blood vessels by means of a growth or sprouting of their endothelial walls, so that, in some cases, an increase in their extent, through growth, may actually take place. It is therefore quite probable that growth may play a considerable rôle in establishing a concrescence between the independent endothelial-lined anlagen of the blood-vascular system. *From whatever standpoint it may be considered, however, the growth of an endothelium is a feature of secondary significance as regards the problem at hand, since the main question at issue does not concern the possibility that endothelium may or may not grow, but rather how the endothelium is formed that does the growing.*

The distinction between the actual genesis of endothelium and the growth it may undergo after it has once been formed is naturally one that has been disregarded by those who maintain that intra-embryonic vascular endothelium is not directly a local product of mesenchymal cells. A special specificity has therefore been attributed by the supporters of the 'angioblast' theory to the endothelium of the intra-embryonic vascular system, on the ground that it takes its origin only from the yolk-sac angioblast. In accordance with this view, it is by means of one continuous and uninterrupted growth of a preexisting endothelium (yolk-sac angioblast) throughout the body of the embryo, that the endothelium of the blood-vascular and lymphatic systems is formed.

Since the 'angioblast' theory of His no longer holds, the question of the specificity of tissues is involved in the vascular problem only to the same extent as is the case for any other tissue in the body. Whether the mesenchymal cells of the embryo are in an embryonic or undifferentiated state, and capable of further differentiation into cells which form muscle, connective tissue, endothelium, etc., is entirely beside the question; provided we know that the product of these intra-embryonic mesenchymal cells actually forms endothelium and that the latter is not de-

rived from the yolk-sac angioblast. Also, the question concerning the origin of these mesenchymal cells, whether derived from entoderm, mesoderm or mesothelium (Bremer '14), does not concern us here. *The main point at issue is the establishment of the fact that the endothelium of the intra-embryonic haemal vessels is the product of a local in situ differentiation of certain cells in the embryo which have not been derived from the yolk-sac angioblast.*

Let us now compare these conditions of the intra-embryonic blood-vascular system, as determined by sections and experiment, with those of the embryonic lymphatic system.

Our knowledge of the embryonic lymphatic system is gradually approaching a state where, in such forms as teleosts and amphibia, it may also be possible to determine by experiment exactly how the lymphatic system is formed. A thorough knowledge of the lymphatic channels and the order of their appearance in the normal embryo would be quite essential, however, before experiment could be successfully applied. Since the anlagen of the lymphatics do not make their appearance in the embryo under normal conditions until after the veins have been established and have begun to function, it is quite possible, in cases of arrested development of the venous system, as demonstrated by Stockard in *Fundulus*, that development might never be successfully carried to the lymphatic stage. Be this as it may, until the problem has been tested by experiment, our knowledge and interpretation of lymphatic development must, for the present, be based upon the observation of fixed and of living material, and its comparison with the known developmental stages of the blood-vascular system, as observed in fixed and in living material, and as verified by experimental means. If it can be shown that the anlagen of the lymphatic system present exactly the same conditions in fixed and in living material, as those of the blood-vascular system, it is reasonable to infer that in their development the lymphatic and blood-vascular systems follow exactly the same genetic plan. *If one were to observe that in certain cases intra-embryonic blood vessels were formed in the living embryo by a sprouting or growth of a preexisting endothelium,*

would he now be justified in claiming that all of the remaining blood vessels of the embryo were formed in the same manner? In view of the fact that we now know that intra-embryonic blood vessels are not all formed in this manner, it would seem that a similar interpretation might also apply to the lymphatics (see E. R. Clark, '11b).

Whatever else the case may be, in view of the above-mentioned experimental investigations of Hahn, Miller and McWhorter, Reagan, and Stockard, it can now be definitely stated that the endothelium of the lymphatic system is neither directly nor indirectly a product of the yolk-sac angioblast. Such being the case, it must either arise *in situ*, like the endothelium of the intra-embryonic veins, from cells other than from a preexisting endothelium; or, be a product entirely of the endothelium of the veins. If the former case be true, the endothelium of the lymphatic system should present exactly the same independent and discontinuous method of origin in the embryo as that of the extra- and intra-embryonic haemal vessels; and, if the development of the lymphatics were followed by the injection method, the same restrictions as regards the injectibility of its independent anlagen should also necessarily apply. On the other hand, if the lymphatic system is entirely a product of the endothelium of the veins, its origin from mesenchyme should naturally never occur. *As a matter of fact, since intra-embryonic vascular endothelium has been shown by experiment to be a local product of mesenchyme, there now remains no valid reason or significance in the claim, as regards its specificity, that lymphatic endothelium is solely a product of that of the veins.*

Let us examine the evidence at hand and see whether the endothelium of the lymphatics, like that of the haemal vessels, develops *in situ* in the mesenchyme, or whether it forms an exception to that of the haemal vessels, and sprouts throughout the body of the embryo from an endothelium (venous) already formed.

It is not the purpose of the present discussion to give an historical review of the literature bearing upon the development of the lymphatic system but merely, on the basis of comparison, to call attention to the evidence in favor of the view that the

lymphatics, like the haemal vessels, are formed by a confluence of independent and discontinuous anlagen, and that their endothelium arises *in situ* from intra-embryonic mesenchymal cells.

A principal contention of Huntington and McClure ('07a) regarding the development of the lymphatic system has been that its anlagen arise independently and discontinuously in the embryo, and that its endothelium does not spread continuously and uninterruptedly throughout the body from the endothelium of the veins. We have repeatedly shown that the lumina of the lymphatics are formed by a confluence of discontinuous and independent lymph vesicles or lymph spaces, and that the cells which constitute the walls of these spaces are derived *in situ* from mesenchyme and not from the endothelium of the veins. In the early stages of our investigations we laid especial stress upon a plan of development for the lymphatic system of mammals which we described under the name of the 'extraintimal' theory of lymphatic development, and which may be briefly described as follows: The development of the thoracic ducts (Huntington, '10, '11a) and mesenteric lymphatics (McClure, '10) in the cat is correlated with the degeneration of certain venous channels, many of which are tributaries of the azygos division of the supracardinal veins (Huntington and McClure, '07b). A series of independent lymph spaces arise discontinuously in the mesenchyme external to the intimal lining of these degenerating vessels and, as these lymph spaces gradually become confluent to form continuous channels, the latter, following a line of least resistance, utilize the static line vacated by these degenerating veins. In this manner certain of the main lymph channels of the mammalian embryo follow the course of and finally occupy completely the territory formerly occupied by veins. This principle of extraintimal replacement of certain evanescent embryonic venous channels by lymphatics accounts for the sinistral drainage plan finally assumed by the thoracic duct system in the embryo of the cat. The cranial or azygos division of the left thoracic duct of the embryo persists as the main line of drainage in the adult, in correlation with a degeneration in the

embryo of the left supracardinal (left azygos) and left postcardinal veins and the left duct of Cuvier.

It is evident and appears clearly in our earlier publications, that the fundamental plan of development followed by these replacing lymph channels does not depart from that followed by other lymph channels, either in mammals or in any other vertebrates where the development of the lymphatics is unaccompanied by the replacement of degenerating veins. Where, as in the case of the trout, lymph channels do not develop along the course of degenerating veins, an extraintimal replacement of a degenerating vein by a lymphatic necessarily does not occur. *It is therefore plain that the extraintimal replacement, as described by us, possesses only a mechanical significance, and is merely an adaptation of a common plan of lymphatic genesis, through the concrescence of independent anlagen, to the local conditions which prevail only in certain districts of the mammalian embryo.*

The same general plan of development as outlined above by Huntington and McClure for the lymphatic system of the cat has also been found by Kampmeier ('12a, '12b) to occur in the embryo of the pig. His description of the independent and discontinuous anlagen of the thoracic ducts which he found in the injected pig embryo loaned him by Professor Sabin, needs no further comment (McClure '12).

F. T. Lewis ('05) has described the presence of a chain of discontinuous 'lymphatic spaces' (endothelial-lined anlagen) in the rabbit embryo which lie along the azygos veins in the path of the future thoracic duct. He regards these anlagen, however, as having been detached from the veins. Concerning these multiple anlagen of Lewis, Sabin ('08) has stated as follows:

Since these spaces are lined with a definite endothelium, they form a much more serious obstacle to the theory of growth of the lymphatics from the endothelium of the veins than the more indefinite spaces to be found in earlier embryos, and I cannot but think that if these multiple endothelial-lined isolated spaces do exist along the veins in later stages, they would form serious evidence against the theory of the origin of the lymphatics from the veins. Or at least if the lymphatics, in their growth, do pick up isolated endothelial-lined

spaces, we shall again be left without a clue as to the origin of the lymphatic system.

It is significant to note that, although Sabin considers these isolated endothelial-lined anlagen of Lewis as having been detached from the veins, she nevertheless now recognizes their existence in the pig embryo, and regards them as entering into the formation of the thoracic duct ('11, p. 424).

The point I wish to emphasize in this connection is that Sabin now recognizes the fact that lymphatics may be formed by the concrescence of multiple and independent endothelial-lined anlagen and that she has thus far presented no valid evidence that these anlagen have been detached from the veins.

Sala ('00) and more recently Miller ('13) have shown that the thoracic ducts of the common fowl are formed by a concrescence of independent and discontinuous lymph spaces and that their endothelium is formed *in situ* from cells other than those which constitute the endothelium of the veins. Miller has further made the important discovery that groups of blood cells develop in the mesenchyme along the line of the thoracic ducts and that the latter subsequently convey these blood cells to the venous circulation. We therefore find that hematopoiesis may actually occur in connection with the development of certain lymph channels, a condition which Huntington ('14) has also recently verified for certain lymphatics of mammals.

West ('14, '15), by a study of injected and uninjected embryos, has recently found that the posterior lymph heart of the common fowl develops in the mesenchyme and secondarily establishes a connection with the veins. He has also found that hematopoiesis occurs in the mesenchyme in relation to the independent anlagen of the lymph heart, and states that the blood cells thus formed are not to be confounded with those which may later back into the lymph heart from the veins (E. L. Clark, '12).

Huntington ('11b), in a paper on the development of the lymphatic system in reptiles (chelonians, lacertilians), has shown that the systemic lymphatics develop in the mesenchyme independently of the endothelium of the veins. A particular feature

of his investigation is that he was able to demonstrate that the periaortic lymph channel in *Chelydra serpentina* arises in the mesenchyme in an area entirely free of veins.

Stromsten's investigations ('10, '11, '12) on the development of the lymphatic system in reptiles (chelonians) have led him to conclude that the lymphatics are formed by a concrescence of independent and discontinuous lymph spaces and that lymphatic endothelium is formed *in situ* from mesenchymal cells. His observations were based largely upon a study of injected embryos, and showed that the injecta did not reach the independent anlagen of the lymphatics, prior to their concrescence with one another to form a system of continuous lumina which had established a communication with the veins.

Fedorowicz ('13) and more recently Kampmeier ('15), have shown that the continuous lumina of certain lymphatics are formed in the amphibia (anura) by the concrescence of originally independent and discontinuous lumina. They conclude, however, that the endothelial walls of these lumina have been derived from the endothelium of the veins. E. R. Clark ('11b) regards the lumina of the developing lymphatics in amphibia (in tail of larval amphibia) as always continuous and capable of injection, while Fedorowicz and Kampmeier describe them as discontinuous at the start.

In the preceding pages the writer has demonstrated the presence of discontinuous and independent lymph vesicles in the trout embryo, which cannot be injected from other lymphatic anlagen or from the veins. Many of these vesicles arise in the mesenchyme remote from the veins, and no connection can be observed between their endothelium and that of the veins or that of any other lymphatic anlagen. One of these independent lymph vesicles, the subocular lymph sac, can actually be observed in the living trout embryo. On account of the relatively large size of this vesicle it has proved a most favorable object for experimentation and for study in sections, not only in proof of the fact that it arises independently of the veins, but also that its endothelium is of mesenchymal origin.

Allen ('13) has investigated the development of the lymphatic system in *Polistotrema* (*Bdellostoma*) *stouti* and speaks of the lymphatics of fishes as veno-lymphatics. He states: "I expect to show that the main factor in the construction of the veno-lymphatic system is the same as was described for the caudal lymph hearts, namely, the formation and union of certain mesenchymal spaces."

Allen, independently of Miller, has also observed that an active hematopoiesis occurs in the mesenchyme in relation to the developing caudal lymph hearts of *Polistotrema*.

Except for differences of opinion regarding the origin of lymphatic endothelium, it may be observed that the above-mentioned investigators agree for the most part that the continuous lumina of the lymphatics, like those of the haemal vessels, are formed by a concrescence of independent and discontinuous anlagen.

If we disregard entirely the personal equation which may have influenced any or all of the above-mentioned investigators to interpret their findings in accordance with one or the other view, the fact still remains that the anlagen of the lymphatic system, as observed in sections of injected and uninjected embryos, have been found to be identical with those of the intra-embryonic blood-vascular system, which has been shown by sections and experiment to be formed by a concrescence of independent anlagen, and its endothelium to be formed *in situ* from mesenchymal cells. *Since we possess exactly the same kind of evidence in favor of the mesenchymal origin of lymphatic endothelium, as we formerly did for that of the intra-embryonic haemal channels, before experiment was applied, it therefore seems highly improbable that the endothelium of two sets of similarly appearing anlagen, belonging to the same general organ system and developing side by side, should differ in its mode of origin, rather than follow a common genetic plan.*

We know that the lymphatics, both in the embryo and in the adult, establish a permanent communication with the veins at typical points (McClure and Silvester '09, and Silvester '12). The question therefore arises, what rôle, if any,

may be played by the veins in establishing such communications with the independently formed lymphatics? In the development of the general vascular system which includes the arteries, veins and lymphatics, the end result desired is the formation of a connected system of vessels which subserve definite functions in the economy of the general vascular system. If the general vascular system develops progressively in a uniform manner, by a concrescence of independently formed anlagen, and the lymphatics, as is the case, form the last link in completing the chain, it is evident that the same factors should account for the establishment of a connection between the veins and the independently formed lymphatics, as between the independently formed anlagen of which the veins and lymphatics are originally composed. Such a connection could alone be established, either by a growth or sprouting of the endothelium of the veins; by means of a further *in situ* differentiation into endothelium of the mesenchymal cells which intervene between the veins and the independent anlagen of the lymphatics; or, both of these factors might be involved. If a connection should be established by a sprouting of the endothelium of the veins, such sprouts would possess no significance beyond the fact which we all recognize, that all vascular endothelium is capable of growth after it has once been formed. The question, however, is not whether endothelium is capable of growth, but rather what are its limitations and how is the endothelium formed that does the growing. It is therefore evident, if, at the points at which the lymphatics establish permanent communications with the veins, venous endothelium should contribute to the formation of the lymphatics, it would play only a subsidiary rôle, and serve only as a means, in common with the endothelium of other independently formed vascular anlagen, of bringing two independently formed portions of the vascular system into communication with each other to form a continuous system of channels. Such veno-lymphatic connections have been hitherto described by Huntington and McClure ('10) under the name of 'venolymphatics.' It is evident, however, that these 'venolymphatics' would not differ, in any sense, from other connections, where a similar growth of

endothelium is concerned, when made for the purpose of establishing a connection between any of the other independently formed anlagen of the general vascular system.

To sum up: The development of the general vascular system—haemal and lymphatic vessels—is a uniform process, which consists in a local origin (genesis) of endothelium from mesenchymal cells and a growth of endothelium after it has once been formed.

In view of what has been said above, it would therefore appear that the lymphatic problem, in its broadest sense, should not be interpreted in terms either of a venous or non-venous origin, but rather in terms of the uniform phases of genesis and growth which may characterize the establishment of vascular channels in general.

One of the principal conclusions arrived at in the course of this investigation, is a further substantiation of the view that the continuous lumina of the lymphatics, like those of the haemal vessels, are formed by a confluence of originally independent and discontinuous vascular spaces; a circumstance which proves that the method of injection throws no light upon the actual mode of origin of the lymphatic system.

The functional rôle played by the independent lymph vesicles of the embryo has already been described by the writer ('15a) in a former paper, to which the reader is referred.

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DESCRIPTION OF FIGURES⁶

The structures shown in the following figures are designated by numerals:

Figures 1 to 13, inclusive, represent reconstructions of the arteries, veins and lymphatics found in the regions of the head and pharynx in the embryos of the trout (figs. 1 to 11, inclusive) and *Amia* (figs. 12 and 13). The arteries are shown in red, the veins in black and the lymphatics in green. The vascular system of the trout embryos (figs. 1 to 11, inclusive) and that of the 10 mm. embryo of *Amia* (fig. 12) were reconstructed after the method of Born, at a magnification of 200 diameters; while that of the 19 mm. embryo of *Amia* (fig. 13) was reconstructed after the method of Born at a magnification of 138 diameters. In order to display the smaller reconstructions to the best advantage, a uniform reduction has not been followed in reducing the size of the figures in the plates.

PLATE 1

EXPLANATION OF FIGURES

Figs. 1-2 Lateral view of the arteries, veins and lymphatics found on the left and right sides, respectively, of the head and pharynx in a fourteen-day steel head trout embryo; injected embryo. P. E. C.⁷ 696.

REFERENCES

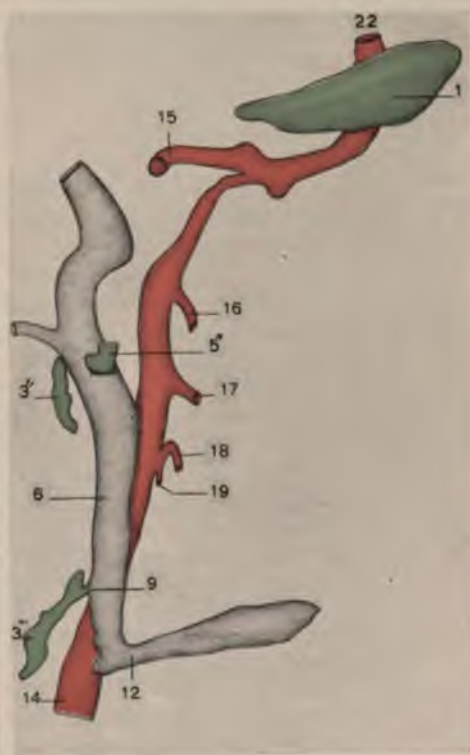
- | | |
|---|---------------------------|
| 1, Subocular lymph sac. | 8', Caudal end of otocyst |
| 3'', Cardio Cuvierian lymph sac; anlage of the lateral pharyngeal lymphatic which may arise opposite to the cardino-Cuvierian junction or between the latter and the otic lymph sac | 11, Postcardinal vein |
| 6, Precardinal or jugular vein | 12, Duct of Cuvier |
| 8, Cranial end of otocyst | 14, Dorsal aorta |
| | 15, Hyoidian artery |
| | 20, Caudal end of eye |
| | 22, Carotid artery |
| | 23, Infraorbital vein |

⁶ The photomicrographs (except fig. 41) were made by Mr. C. F. Silvester, and figures 1 to 13, inclusive, were drawn from reconstructions by Mr. Anthony J. Walter.

⁷ Princeton Embryological Collection.



3



4

PLATE 2

EXPLANATION OF FIGURES

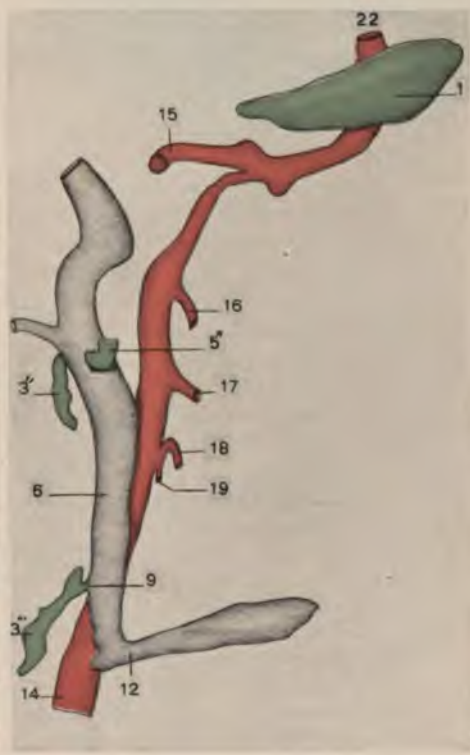
Figs. 3-4 Lateral view of the arteries, veins and lymphatics found on the left and right sides, respectively, of the head and pharynx in a fifteen-day steel-head trout embryo; injected embryo. P. E. C. 726.

REFERENCES

- | | |
|---|--|
| 1, Subocular lymph sac | 9, Cardino-Cuvierian communication; |
| 3'', Otic lymph sac; anlage of the | the point at which the lateral pharyn- |
| lateral pharyngeal lymphatic which | geal lymphatic communicates with |
| lies near the caudal end of the otocyst | the precardinal vein in the cardino- |
| and which may establish a communi- | Cuvierian district |
| cation with the precardinal vein at | 12, Duct of Cuvier |
| the otic communication | 14, Dorsal aorta |
| 3''', Cardino-Cuvierian lymph sac; | 15, Hyoidean artery |
| anlage of the lateral pharyngeal lym- | 16, First efferent aortic arch |
| phatic which lies between the car- | 17, Second efferent aortic arch |
| dino-Cuvierian junction and the otic | 18, Third efferent aortic arch |
| lymph sac | 19, Fourth efferent aortic arch |
| 6, Precardinal or jugular vein | 22, Carotid artery |



3



4

PLATE 3

EXPLANATION OF FIGURE

Fig. 5 Ventral view of the arteries, veins and lymphatics found in the head and pharynx of a sixteen-day steelhead trout embryo; injected embryo. P. E. C. 751.

REFERENCES

- | | |
|---|--|
| 1, Subocular lymph sac | 9, Cardio-Cuvierian communication; the point at which the lateral pharyngeal lymphatic communicates with the precardinal vein in the cardio-Cuvierian district |
| 3', Independent anlage of the lateral pharyngeal lymphatic which lies anterior to the otic communication | 10, Lymphatic of the lateral line of the trunk |
| 3'', Otic lymph sac; anlage of the lateral pharyngeal lymphatic which lies near the caudal end of the otocyst and which may establish a communication with the precardinal vein at the otic communication | 11, Postcardinal vein |
| 3''', Cardio-Cuvierian lymph sac; anlage of the lateral pharyngeal lymphatic which lies opposite to the cardio-Cuvierian junction | 12, Duct of Cuvier |
| 5'', Anlage of the precardinal lymphatic which lies contiguous to the otic communication and which establishes a connection with the lateral pharyngeal lymphatic | 13, Otic communication; the point at which the lateral pharyngeal lymphatic communicates with the precardinal vein near the caudal end of the otocyst |
| 6, Precardinal or jugular vein | 14, Dorsal aorta |
| 7, Point at which the precardinal lymphatic communicates with the lateral pharyngeal lymphatic | 15, Hyoidian artery |
| 8, Cranial end of otocyst | 16, First efferent aortic arch |
| | 17, Second efferent aortic arch |
| | 18, Third efferent aortic arch |
| | 19, Fourth efferent aortic arch |
| | 20, Caudal end of eye |
| | 22, Carotid artery |
| | 23, Infraorbital vein |
| | 24, Cardio-Cuvierian junction |
| | 27, Root of dorsal aorta |

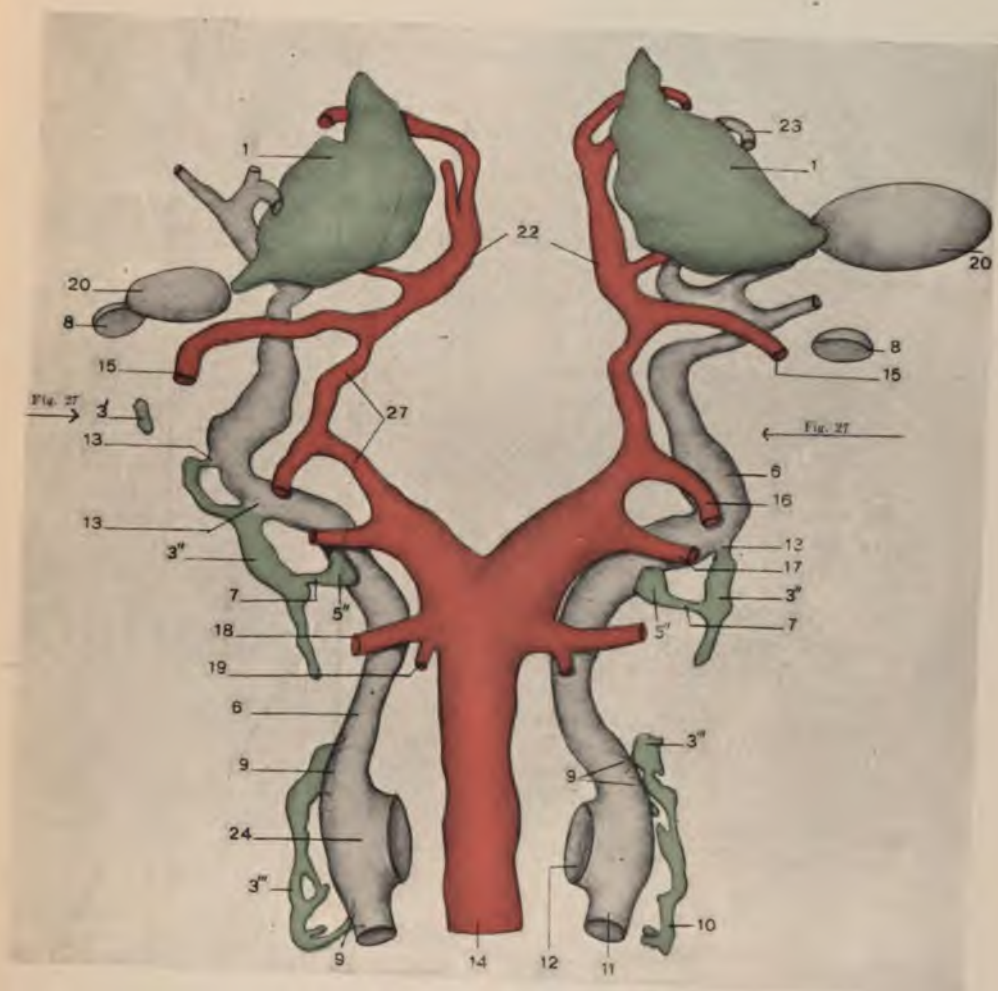


PLATE 4

EXPLANATION OF FIGURE

Fig. 6 Ventral view of the arteries, veins and lymphatics found in the head and pharynx of a seventeen-day steelhead trout embryo; injected embryo. P. E. C. 740.

REFERENCES

- | | |
|---|--|
| 1, Subocular lymph sac | 6, Precardinal or jugular vein |
| 3', Independent anlagen of the lateral pharyngeal lymphatic which lie anterior to the otic communication | 8, Cranial end of otocyst |
| 3'', Otic lymph sac; anlage of the lateral pharyngeal lymphatic which lies near the caudal end of the otocyst and which may establish a communication with the precardinal vein at the otic communication | 9, Cardio-Cuvierian communication; the point at which the lateral pharyngeal lymphatic communicates with the precardinal vein in the cardio-Cuvierian district |
| 3''', Cardio-Cuvierian lymph sac; anlage of the lateral pharyngeal lymphatic which lies opposite to the cardio-Cuvierian junction and between the latter and the otic lymph sac | 10, Lymphatic of the lateral line of the trunk |
| 5'', Anlage of the precardinal lymphatic which lies contiguous to the otic communication and which establishes a connection with the lateral pharyngeal lymphatic | 14, Dorsal aorta |
| | 15, Hyoidean artery |
| | 16, First efferent aortic arch |
| | 17, Second efferent aortic arch |
| | 18, Third efferent aortic arch |
| | 19, Fourth efferent aortic arch |
| | 20, Caudal end of eye |
| | 22, Carotid artery |
| | 24, Cardio-Cuvierian junction |
| | 27, Root of dorsal aorta |

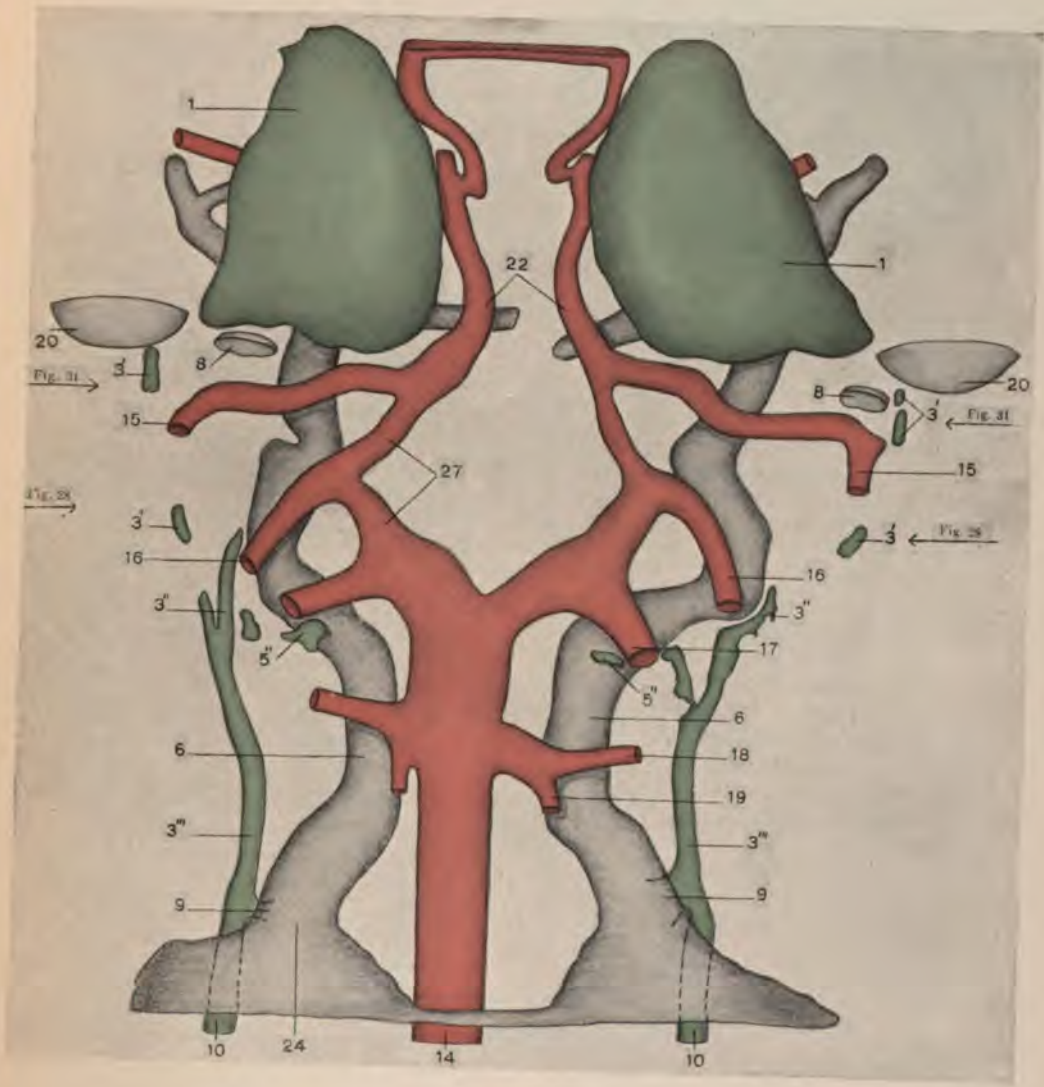


PLATE 5

EXPLANATION OF FIGURE

Fig. 7. Ventral view of the arteries, veins and lymphatics found in the head and pharynx of a nineteen-day rainbow trout embryo; injected embryo. P. E. C. 672.

REFERENCES

- | | |
|---|--|
| 1, Subocular lymph sac | 6, Precardinal or jugular vein |
| 3', Independent anlagen of the lateral pharyngeal lymphatic which lie anterior to the otic communication | 8, Cranial end of otocyst |
| 3'', Otic lymph sac; anlage of the lateral pharyngeal lymphatic which lies near the caudal end of the otocyst and which may establish a communication with the precardinal vein at the otic communication | 9, Cardino-Cuvierian communication; the point at which the lateral pharyngeal lymphatic communicates with the precardinal vein in the cardino-Cuvierian district |
| 3''', Cardino-Cuvierian lymph sac; anlage of the lateral pharyngeal lymphatic which lies opposite to the cardino-Cuvierian junction and between the latter and the otic lymph sac | 10, Lymphatic of the lateral line of the trunk |
| 5'', Anlage of the precardinal lymphatic which lies contiguous to the otic communication and which establishes a connection with the lateral pharyngeal lymphatic | 14, Dorsal aorta |
| 5''', Independent anlage of the precardinal lymphatic which lies, approximately, opposite to the origin of the third efferent aortic arch | 15, Hyoidian artery |
| | 16, First efferent aortic arch |
| | 17, Second efferent aortic arch |
| | 18, Third efferent aortic arch |
| | 19, Fourth efferent aortic arch |
| | 20, Caudal end of eye |
| | 22, Carotid artery |
| | 27, Root of dorsal aorta |
| | 31, Independent anlagen of a lymphatic which arises along the course of the hyoidian artery; these anlagen have been omitted in most of the reconstructions |

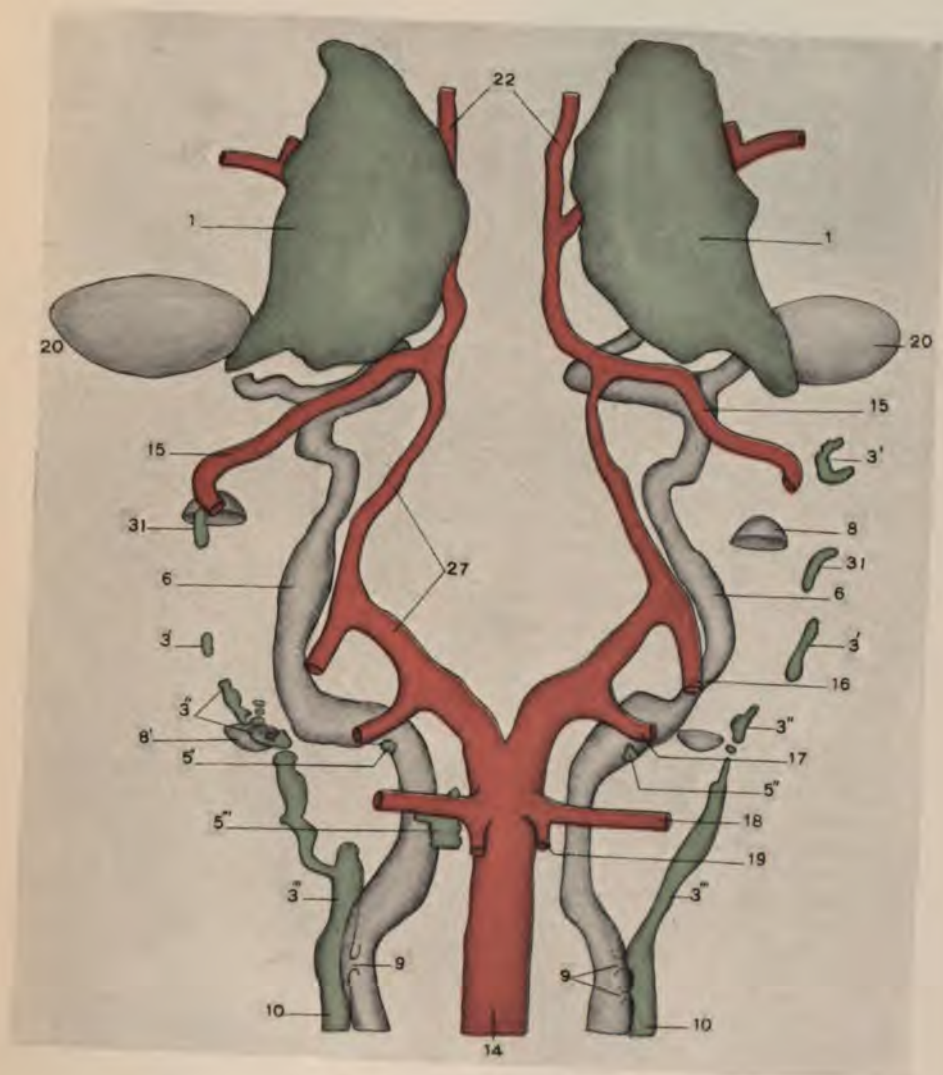


PLATE 6

EXPLANATION OF FIGURE

Fig. 8. Ventral view of the arteries, veins and lymphatics found in the head and pharynx of a twenty-day rainbow trout embryo; injected embryo. P. E. C. 668.

REFERENCES

- | | |
|---|--|
| 1, Subocular lymph sac | 9, Cardio-Cuvierian communication; the point at which the lateral pharyngeal lymphatic communicates with the precardinal vein in the cardio-Cuvierian district |
| 3', Independent anlagen of the lateral pharyngeal lymphatic which lie anterior to the otic communication | 10, Lymphatic of the lateral line of the trunk |
| 3'', Otic lymph sac; anlage of the lateral pharyngeal lymphatic which lies near the caudal end of the otocyst and which may establish a communication with the precardinal vein at the otic communication | 11, Postcardinal vein |
| 3''', Cardio-Cuvierian lymph sac; anlage of the lateral pharyngeal lymphatic which lies opposite to the cardio-Cuvierian junction and between the latter and the otic lymph sac | 14, Dorsal aorta |
| 5'', Anlage of the precardinal lymphatic which lies contiguous to the otic communication and which establishes a connection with the lateral pharyngeal lymphatic | 15, Hyoidean artery |
| 6, Precardinal or jugular vein | 16, First efferent aortic arch |
| 8, Cranial end of otocyst | 17, Second efferent aortic arch |
| | 18, Third efferent aortic arch |
| | 19, Fourth efferent aortic arch |
| | 20, Caudal end of eye |
| | 22, Carotid artery |
| | 27, Root of dorsal aorta |
| | 31, Independent anlage of a lymphatic which arises along the course of the hyoidean artery; these anlagen have been omitted in most of the reconstructions |

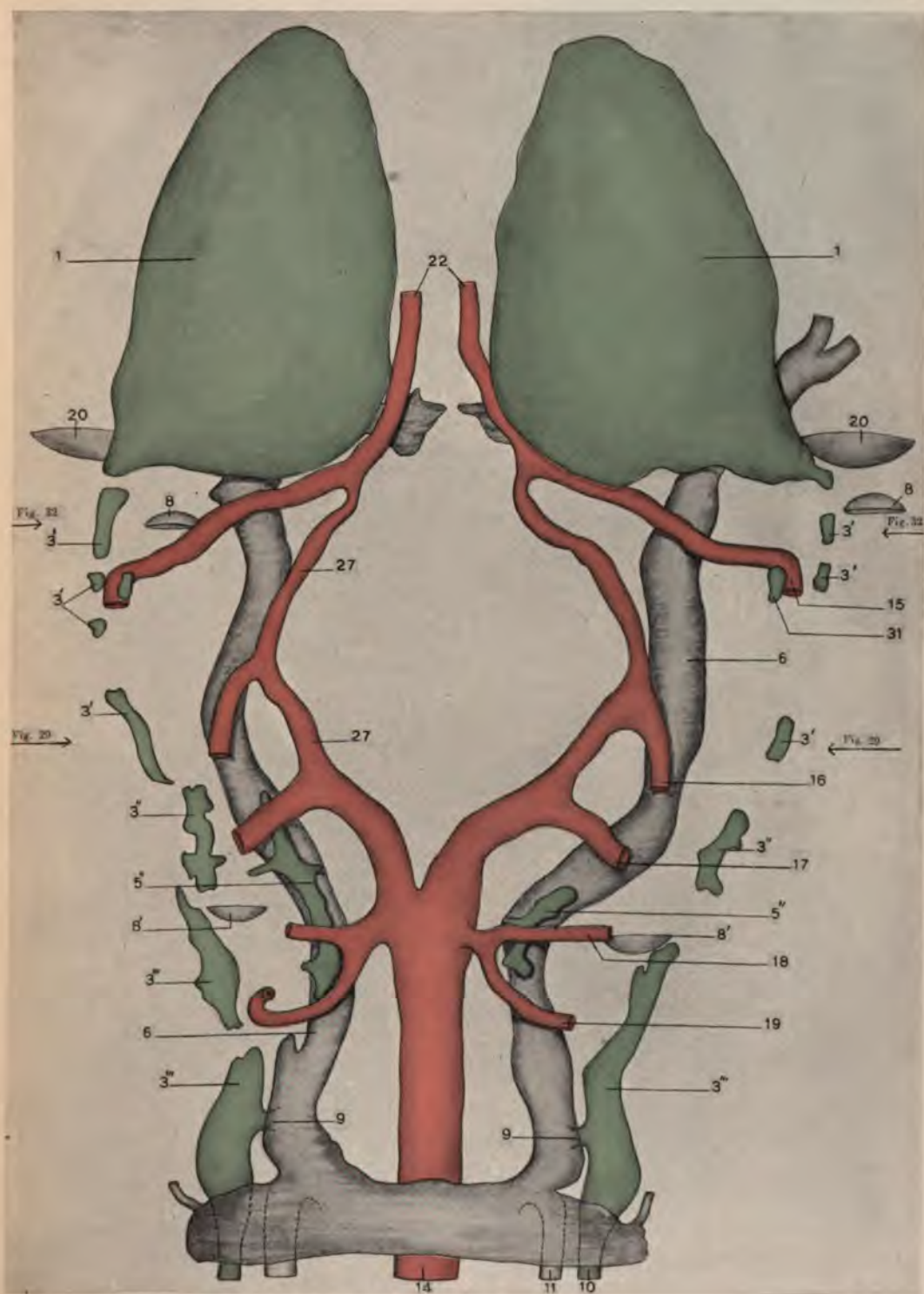


PLATE 7

EXPLANATION OF FIGURE

Fig. 9 Ventral view of the arteries, veins and lymphatics found in the head and pharynx of a twenty-one-day rainbow trout embryo; injected embryo. P. E. C. 646.

REFERENCES

- | | |
|---|--|
| 1, Subocular lymph sac | opposite to the origin of the third efferent aortic arch |
| 2, Medial pharyngeal communication; the point at which the medial pharyngeal lymphatic communicates with the precardinal vein | 6, Precardinal or jugular vein |
| 3', Independent anlagen of the lateral pharyngeal lymphatic which lie anterior to the otic communication | 7, Point at which the precardinal lymphatic communicates with the lateral pharyngeal lymphatic |
| 3'', Otic lymph sac; anlage of the lateral pharyngeal lymphatic which lies near the caudal end of the otocyst and which may establish a communication with the precardinal vein at the otic communication | 9, Cardino-Cuvierian communication; the point at which the lateral pharyngeal lymphatic communicates with the precardinal vein in the cardino-Cuvierian district |
| 3''', Cardino-Cuvierian lymph sac; anlage of the lateral pharyngeal lymphatic which lies opposite to the cardino-Cuvierian junction and between the latter and the otic lymph sac | 10, Lymphatic of the lateral line of the trunk |
| 4, Medial pharyngeal lymphatic | 11, Postcardinal vein |
| 5'', Anlage of the precardinal lymphatic which lies contiguous to the otic communication and which establishes a connection with the lateral pharyngeal lymphatic | 13, Otic communication; the point at which the lateral pharyngeal lymphatic communicates with the precardinal vein near the caudal end of the otocyst |
| 5''', Anlage of the precardinal lymphatic which lies, approximately, | 15, Hyoid artery |
| | 16, First efferent aortic arch |
| | 17, Second efferent aortic arch |
| | 18, Third efferent aortic arch |
| | 19, Fourth efferent aortic arch |
| | 22, Carotid artery |
| | 27, Root of dorsal aorta |
| | 31, Independent anlage of a lymphatic which arises along the course of the hyoid artery |

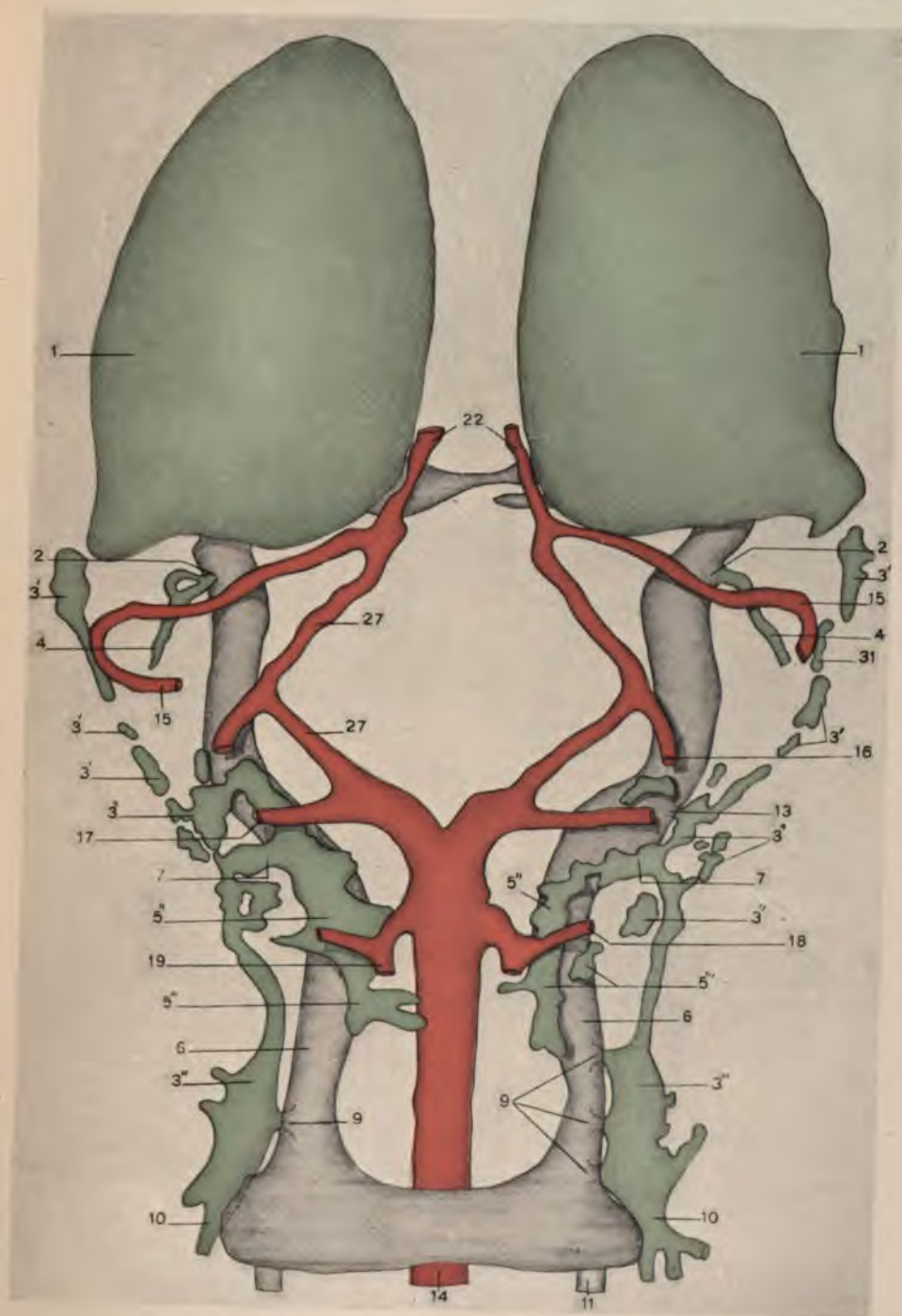


PLATE 8

EXPLANATION OF FIGURE

Fig. 10 Ventral view of the arteries, veins and lymphatics found in the head and pharynx of a twenty-two-day rainbow trout embryo; not injected. P. E. C. 648.

REFERENCES

- | | |
|--|---------------------------------------|
| 1, Subocular lymph sac | geal lymphatic communicates with |
| 2, Medial pharyngeal communication; | the precardinal vein in the cardino- |
| the point at which the medial pharyn- | Cuvierian district |
| geal lymphatic communicates with | 10, Lymphatic of the lateral line of |
| the precardinal vein | the trunk |
| 3, The lateral pharyngeal lymphatic, | 11, Postcardinal vein |
| a continuous vessel through which | 12, Duct of Cuvier |
| the subocular lymph sac drains into | 13, Otic communication; the point |
| the veins in the trout | at which the lateral pharyngeal lym- |
| 4, Medial pharyngeal lymphatic | phatic communicates with the precar- |
| 5, Preadinal or jugular lymphatic | dinal vein near the caudal end of the |
| 5', Independent anlage of the pre- | otocyst |
| cardinal lymphatic which lies con- | 14, Dorsal aorta |
| tiguous to the precardinal vein and | 15, Hyoidean artery |
| just caudal to the medial pharyn- | 16, First efferent aortic arch |
| geal communication | 17, Second efferent aortic arch |
| 6, Preadinal or jugular vein | 18, Third efferent aortic arch |
| 7, Point at which the precardinal lym- | 19, Fourth efferent aortic arch |
| phatic communicates with the lateral | 20, Caudal end of eye |
| pharyngeal lymphatic | 22, Carotid artery |
| 9, Cardino-Cuvierian communication; | 24, Cardino-Cuvierian junction |
| the point at which the lateral pharyn- | 27, Root of dorsal aorta |

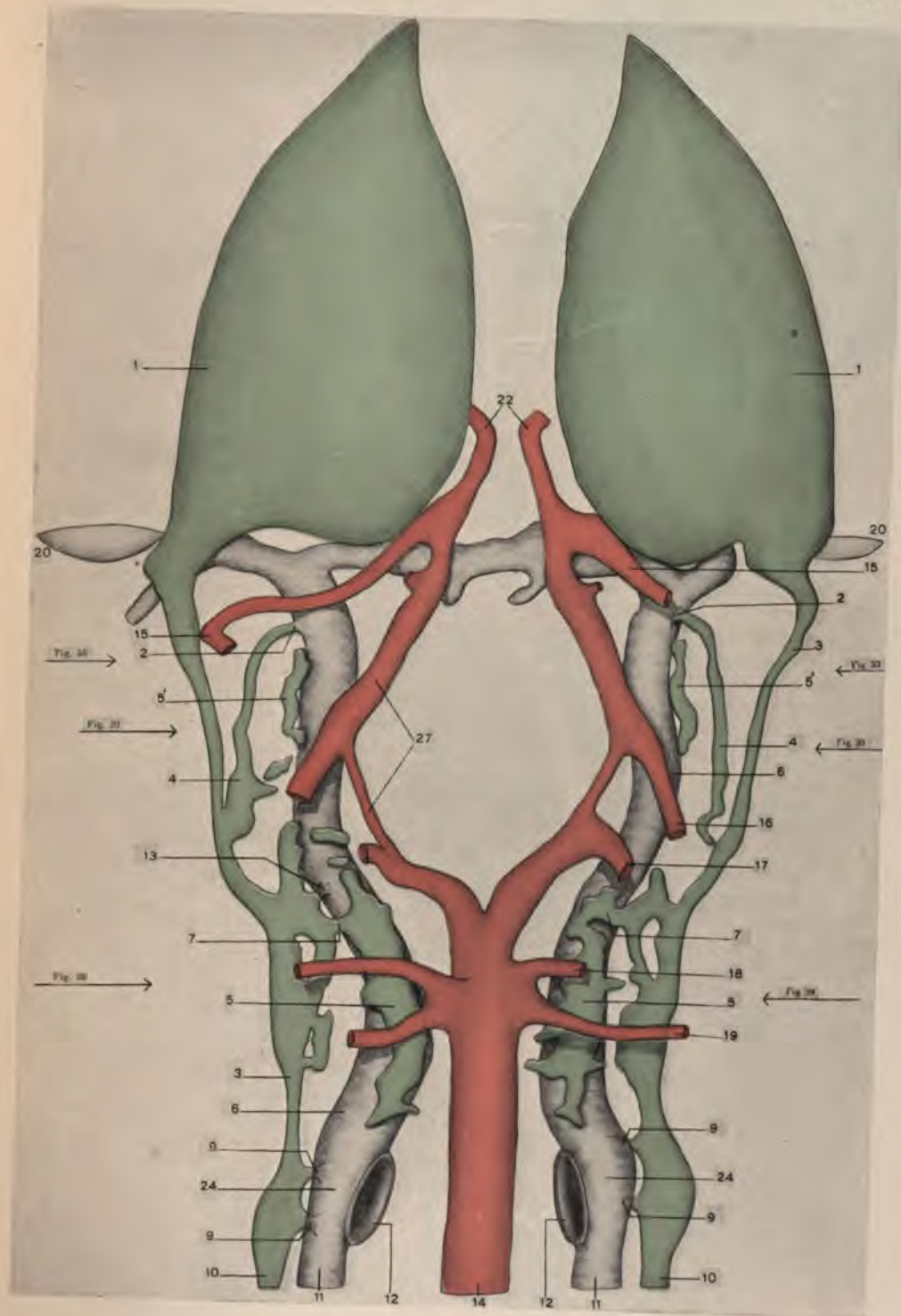


PLATE 9

EXPLANATION OF FIGURE

Fig. 11 Ventral view of the arteries, veins and lymphatics found on the left side of the head and pharynx in a 17.5 mm. brook trout embryo. P. E. C. 393.

REFERENCES

- | | |
|--|--------------------------------------|
| 1, Subocular lymph sac | 13, Otic communication; the point at |
| 2, Medial pharyngeal communication; | which the lateral pharyngeal lym- |
| the point at which the medial pharyn- | phatic communicates with the pre- |
| geal lymphatic communicates with | cardinal vein near the caudal end of |
| the precardinal vein | the otocyst |
| 3, The lateral pharyngeal lymphatic | 14, Dorsal aorta |
| 4, Medial pharyngeal lymphatic | 15', Efferent pseudobranchial artery |
| 5, Precardinal or jugular lymphatics | (ophthalmic artery) |
| 6, Precardinal or jugular vein | 16, First efferent aortic arch |
| 9, Cardio-Cuvierian communication; | 17, Second efferent aortic arch |
| the point at which the lateral pharyn- | 18, Third efferent aortic arch |
| geal lymphatic communicates with | 19, Fourth efferent aortic arch |
| the precardinal vein in the cardino- | 21, Superficial facial lymphatic |
| Cuvierian district | 22, Carotid artery |
| 10, Lymphatic of the lateral line of the | 30, Pericardial lymphatic |
| trunk | |

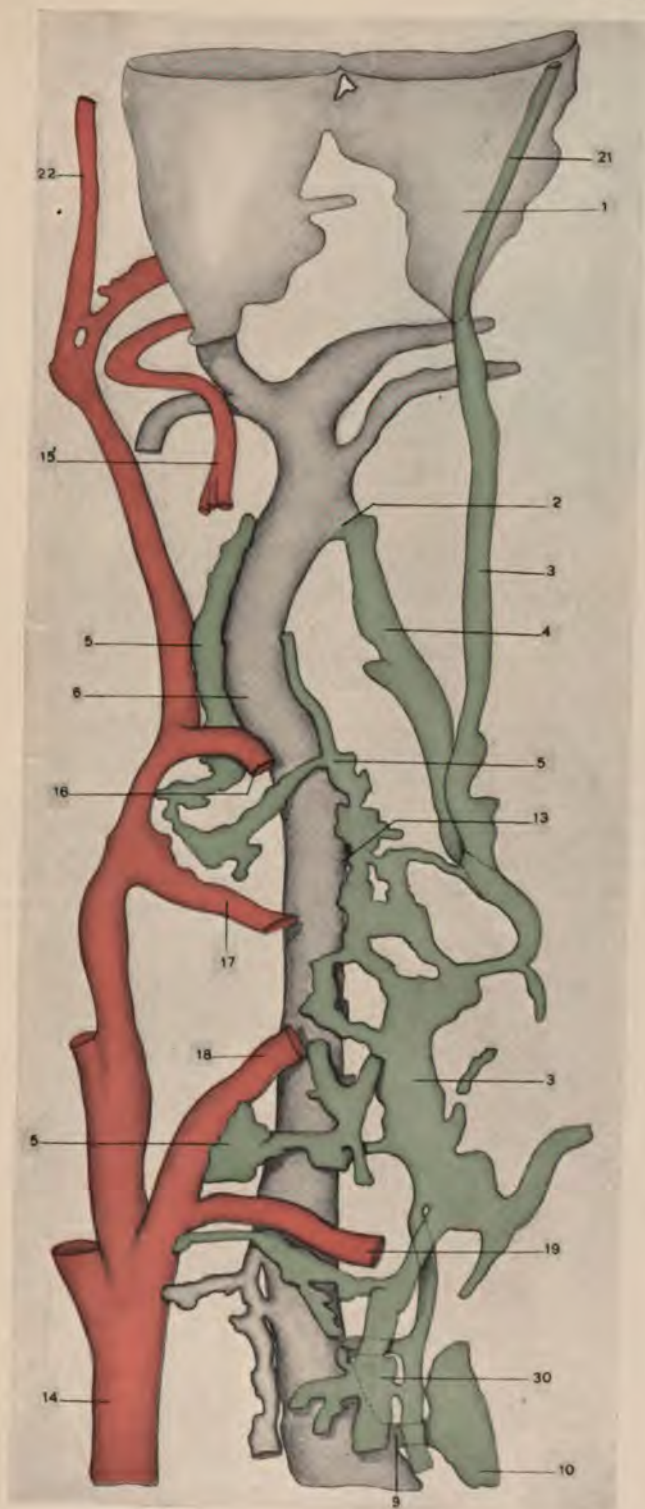


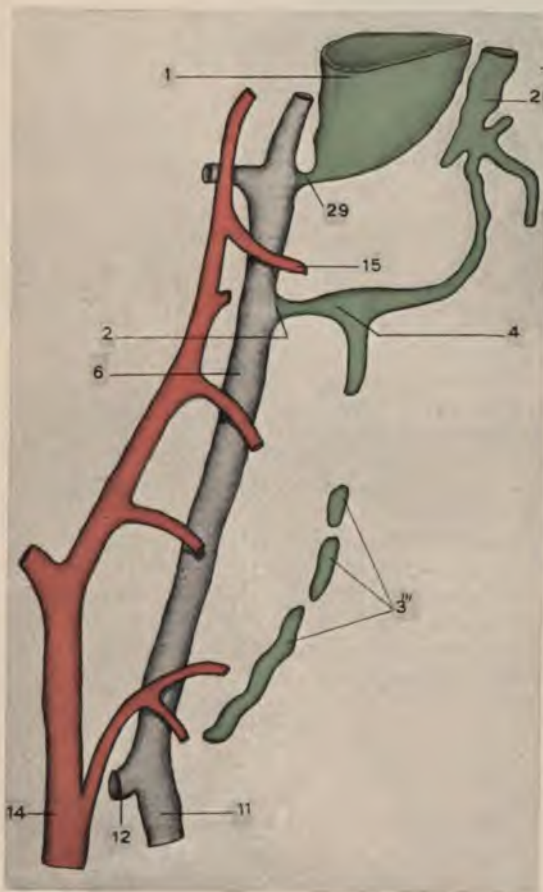
PLATE 10

EXPLANATION OF FIGURE

Fig. 12 Ventral view of the arteries, veins and lymphatics found on the left side of the head and pharynx in a 10 mm. embryo of *Amia calva*; semi-diagrammatic. P. E. C. 447.

REFERENCES

- | | |
|--|---|
| 1, Subocular lymph sac | 11, Postcardinal vein |
| 2, Medial pharyngeal communication;
the point at which the medial pharyngeal lymphatic communicates with the precardinal vein | 12, Duct of Cuvier |
| 3'', Independent anlagen of the lateral pharyngeal lymphatic | 14, Dorsal aorta |
| 4, Medial pharyngeal lymphatic | 15, Hyoidean artery |
| 6, Precardinal or jugular vein | 21, Superficial facial lymphatic |
| | 29, Temporary connection established between the subocular lymph sac and the veins in the embryos of <i>Amia</i> and <i>Lepidosteus</i> |



12

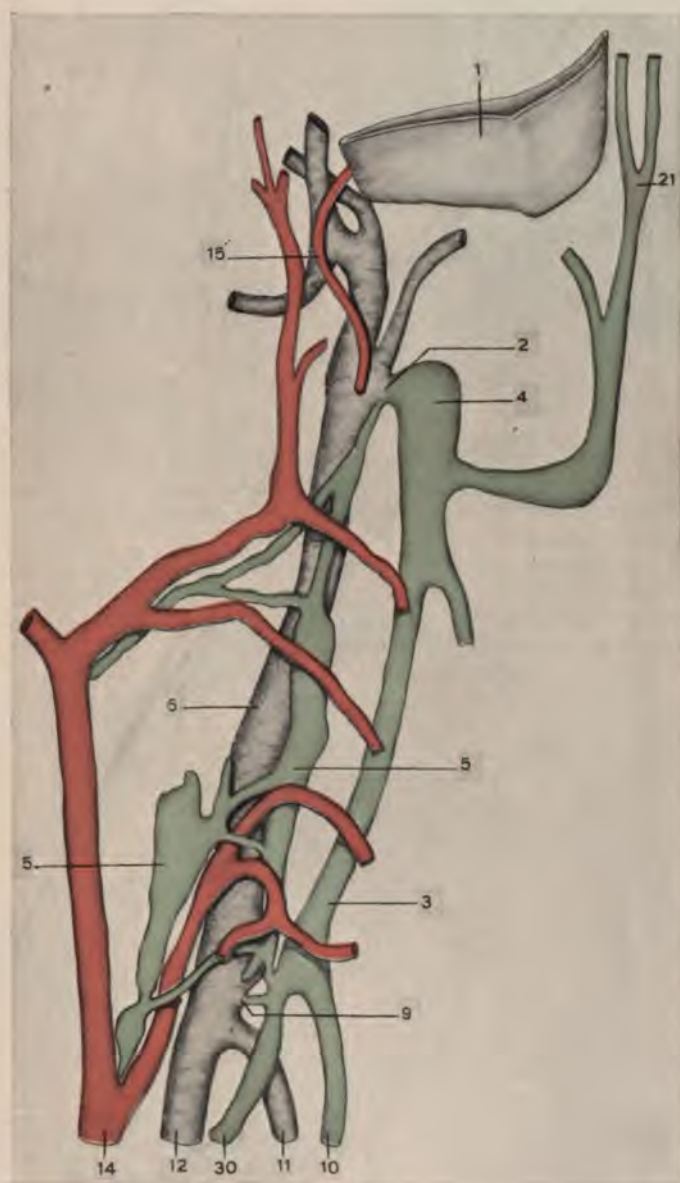
PLATE 11

EXPLANATION OF FIGURE

Fig. 13 Ventral view of the arteries, veins and lymphatics found on the left side of the head and pharynx in a 19 mm. embryo of *Amia calva*; semi-diagrammatic. P. E. C. 387.

REFERENCES

- | | |
|--|--|
| 1, Subocular lymph sac | geal lymphatic communicates with |
| 2, Medial pharyngeal communication; | the precardinal vein in the cardino- |
| the point at which the medial pharyn- | Cuvierian district |
| geal lymphatic communicates with | 10, Lymphatic of the lateral line of the |
| the precardinal vein | trunk |
| 3, The lateral pharyngeal lymphatic | 11, Postcardinal vein |
| 4, Medial pharyngeal lymphatic | 12, Duct of Cuvier |
| 5, Precardinal or jugular lymphatics | 14, Dorsal aorta |
| 6, Precardinal or jugular vein | 15', Efferent pseudobranchial artery |
| 9, Cardio-Cuvierian communication; | 21, Superficial facial lymphatic |
| the point at which the lateral pharyn- | 30, Pericardial lymphatic |



13

97

PLATE 12

EXPLANATION OF FIGURES

Fig. 14 Photomicrograph of a sagittal section taken through the head and pharynx of a twenty-two-day rainbow trout embryo; showing the relations of the subocular lymph sac (1) to the eye, the hyoidean artery (15) and the olfactory pit (33); injected embryo. P. E. C. 816.

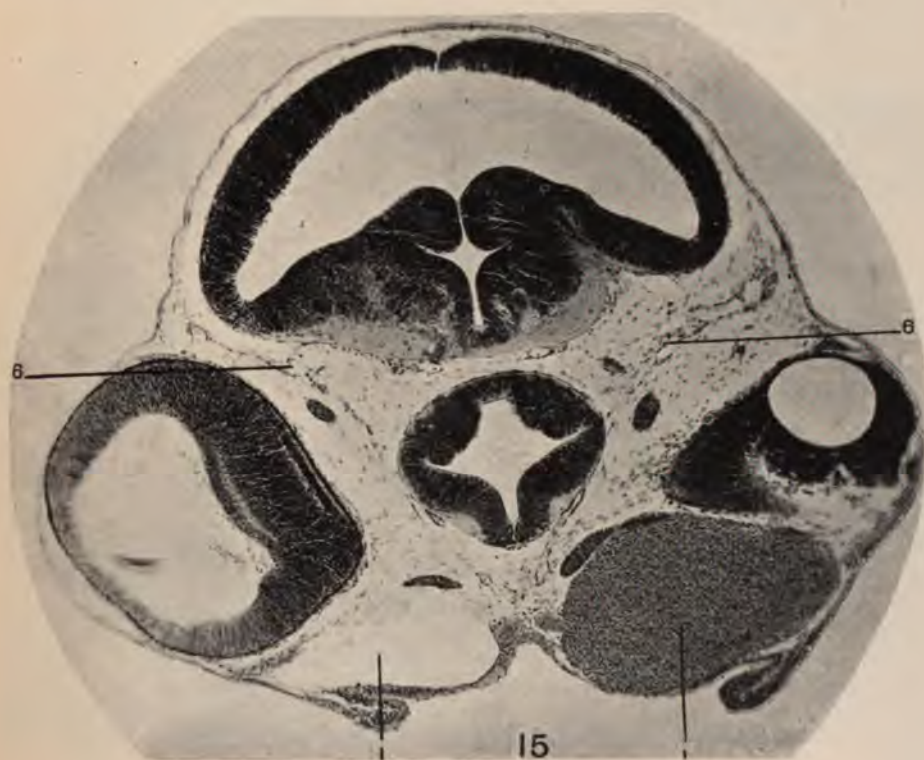
Fig. 15 Photomicrograph of a transverse section taken through the subocular lymph sacs of a twenty-day steelhead trout embryo. The subocular sac (1) on the right side of the figure is filled with blood, which has passed into the sac from the veins through the lateral pharyngeal lymphatic. P. E. C. 636, slide 1, section 67.

REFERENCES

- | | |
|--------------------------------|---------------------|
| 1, Subocular lymph sac | 15, Hyoidean artery |
| 6, Precardinal or jugular vein | 33, Olfactory pit |



14



99

PLATE 13

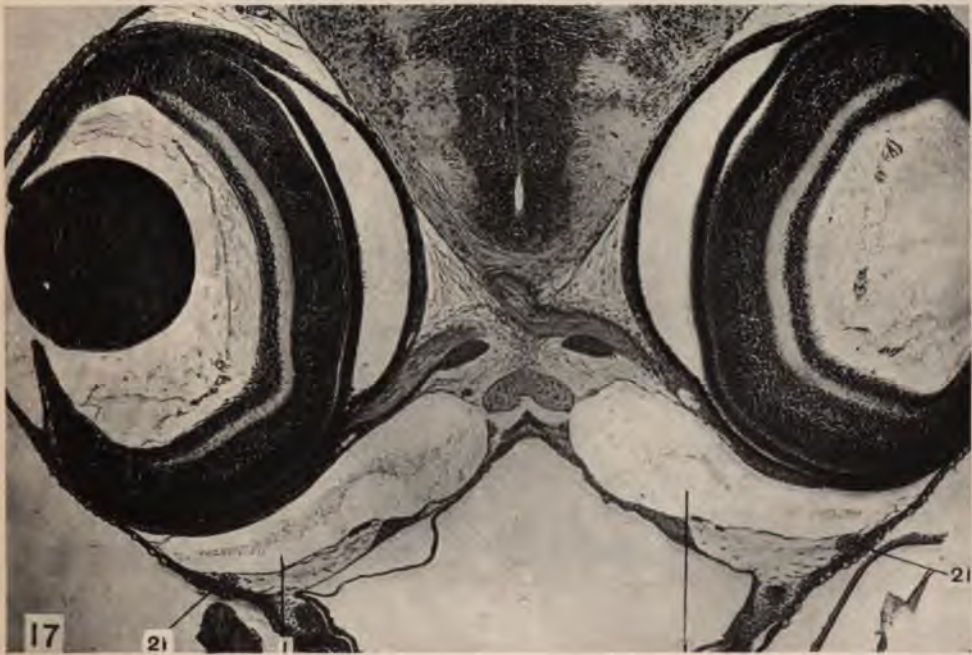
EXPLANATION OF FIGURES

Fig. 16 Photomicrograph of a transverse section taken through the caudal end of the right subocular lymph sac (1) of an 18.25 mm. rainbow trout embryo in which the subocular sac has become detached from the lateral pharyngeal lymphatic (3). The lateral pharyngeal lymphatic is filled with blood. P. E. C. 944, slide 1, section 134.

Fig. 17 Photomicrograph of a transverse section taken through the subocular lymph sacs (1) of an 18.25 mm. rainbow trout embryo at the level of the optic nerves, showing the position occupied by the superficial facial lymphatic (21) which is filled with blood. P. E. C. 944, slide 1, section 78.

REFERENCES

- | | |
|-------------------------------------|----------------------------------|
| 1, Subocular lymph sac | 6, Precardinal or jugular vein |
| 3, The lateral pharyngeal lymphatic | 21, Superficial facial lymphatic |



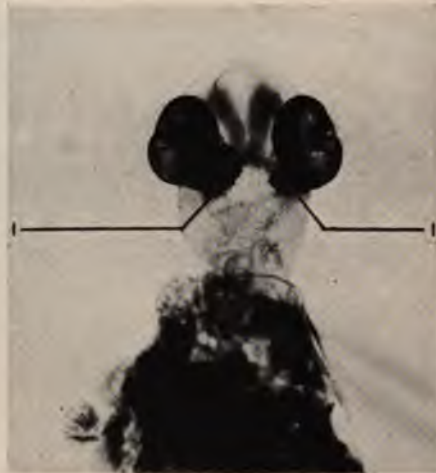


Fig. 18 Photograph of the ventral aspect of the head of a twenty-day rainbow trout embryo on which an attempt was made to inject the lymphatics and the veins by injecting into the subocular lymph sacs (1); Spalteholz preparation.

PLATE 14

EXPLANATION OF FIGURE

Fig. 19 Photomicrograph of a frontal section taken through the anlagen of the subocular lymph sacs (1) in a fourteen-day steelhead trout embryo. P. E. C. 697, slide 1, section 69; injected embryo.

REFERENCES

- | | |
|--------------------------------|--------------------|
| 1, Subocular lymph sac | 22, Carotid artery |
| 6, Precardinal or jugular vein | 28, Infundibulum |



PLATE 15

EXPLANATION OF FIGURE

Fig. 20 Photomicrograph of a frontal section taken through the anlagen of the subocular lymph sacs (1) in a fourteen-day steelhead trout embryo. P. E. C. 696, slide 1, section 69; injected embryo.

REFERENCES

- | | |
|--------------------------------|--------------------|
| 1, Subocular lymph sac | 22, Carotid artery |
| 6, Precardinal or jugular vein | 28, Infundibulum |

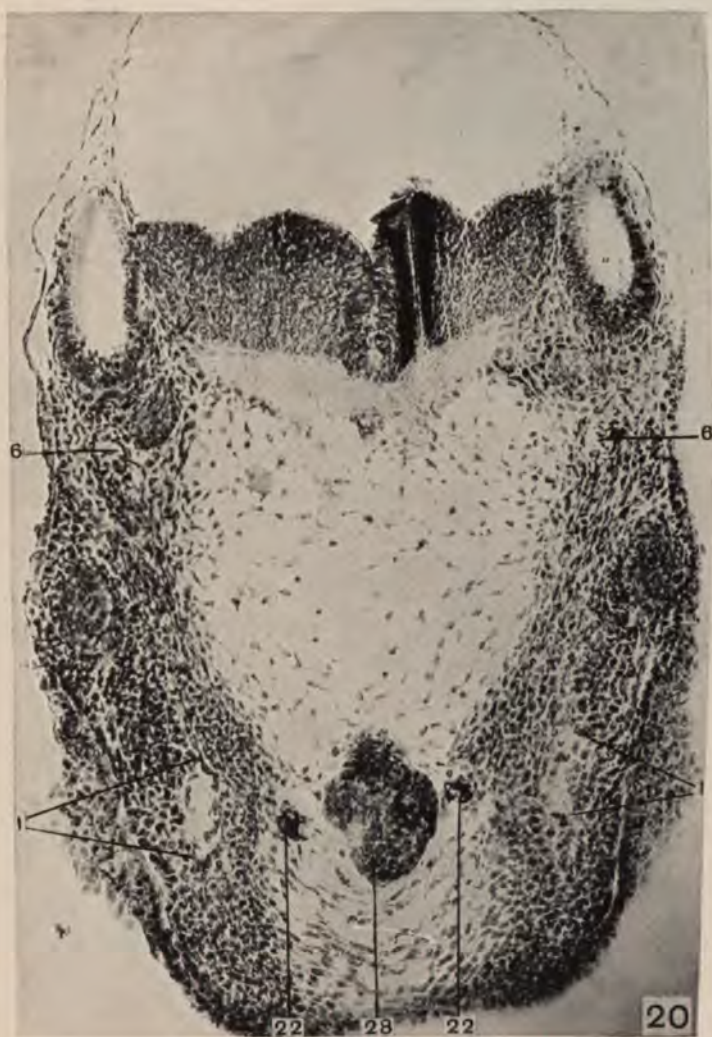


PLATE 16

EXPLANATION OF FIGURE

Fig. 21 Photomicrograph of a transverse section taken through the subocular lymph sac (1) in a seventeen-day steelhead trout embryo in which the sac presents a multilocular appearance; high magnification. P. E. C. 522, slide 1, section 95; injected embryo.

REFERENCES

1, Subocular lymph sac

22, Carotid artery

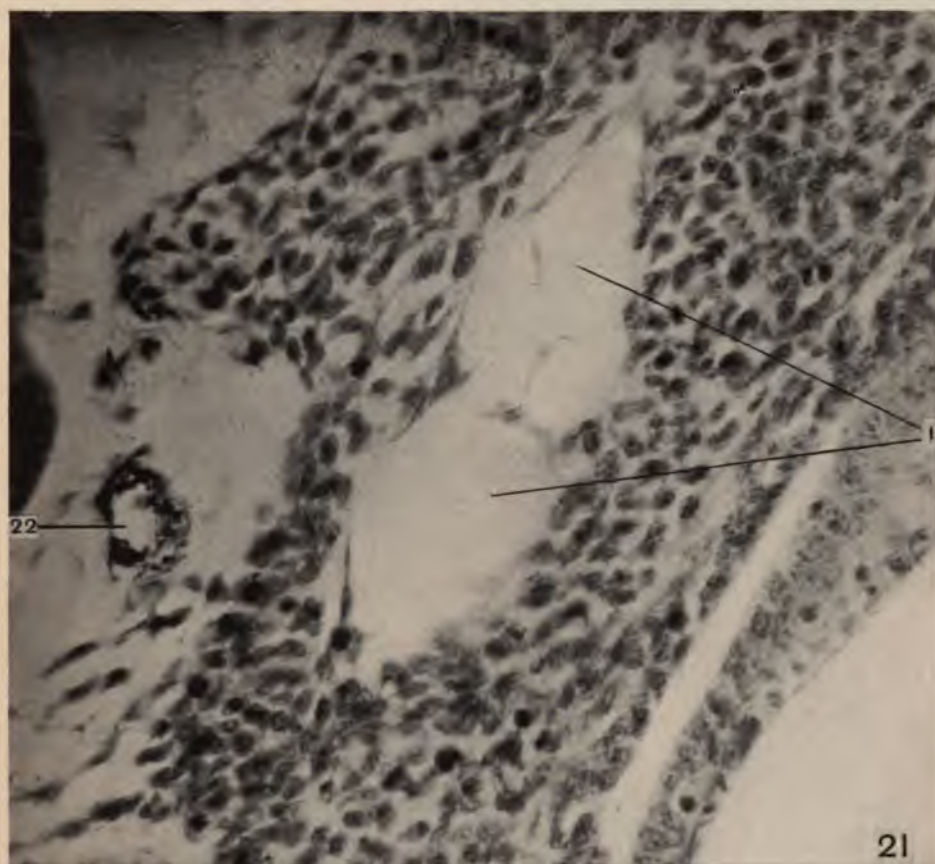


PLATE 17

EXPLANATION OF FIGURE

Fig. 22 Photomicrograph of a transverse section taken through the subocular lymph sacs (1) in a fourteen-day steelhead trout embryo. P. E. C. 695, slide 1, section 70; injected embryo.

REFERENCES

- | | |
|--------------------------------|--------------------|
| 1, Subocular lymph sac | 22, Carotid artery |
| 6, Precardinal or jugular vein | 28, Infundibulum |
| 20, Caudal end of eye | |

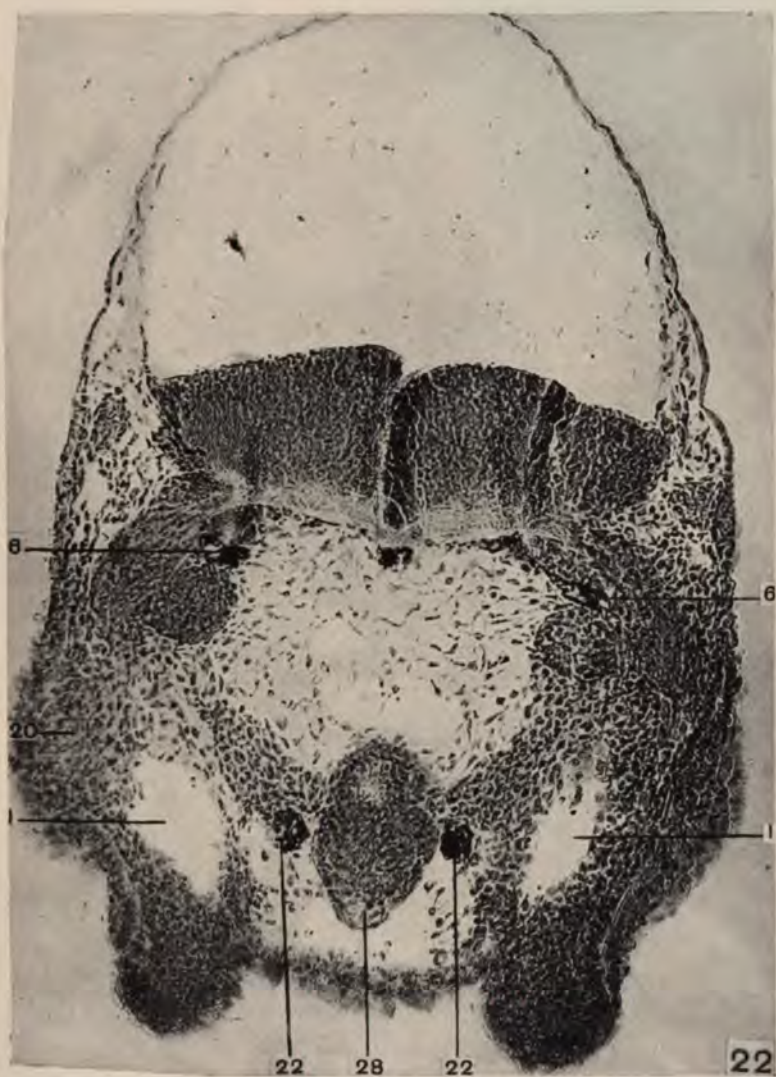


PLATE 18

EXPLANATION OF FIGURES

Fig. 23 Photomicrograph of a transverse section taken through the subocular lymph sacs (1) of a fifteen-day steelhead trout embryo. P. E. C. 726, slide 1, section 78; injected embryo.

Fig. 24 Photomicrograph of a transverse section taken through the subocular lymph sacs (1) of a sixteen-day rainbow trout embryo at the level of the infra-orbital veins (23); showing that the veins are fully injected and that none of the injecta has passed into the sacs. P. E. C. 751, slide 1, section 66.

REFERENCES

- | | |
|--------------------------------|-----------------------|
| 1, Subocular lymph sac | 22, Carotid artery |
| 6, Precardinal or jugular vein | 23, Infraorbital vein |
| 15, Hyoidian artery | 28, Infundibulum |
| 20, Caudal end of eye | |



PLATE 19

EXPLANATION OF FIGURE

• Fig. 25 Photomicrograph of a transverse section of a fourteen-day steelhead trout embryo, taken at the level of the infraorbital veins (23); showing that in the course of their forward extension, the subocular lymph sacs have not reached the level of the infraorbital veins. P. E. C. 696, slide 1, section 61 injected embryo.

REFERENCES

6, Precardinal or jugular vein	23, Infraorbital vein
20, Caudal end of eye	28, Infundibulum
22, Carotid artery	



PLATE 20

EXPLANATION OF FIGURE

Fig. 26 Photomicrograph of a transverse section of a twenty-two-day rainbow trout embryo taken through the anterior end of the left subocular lymph sac (1); showing that the anterior extension of the sac is brought about by a confluence of independent anlagen. P. E. C. 648, slide 1, section 48.

REFERENCES

- 1, Subocular lymph sac

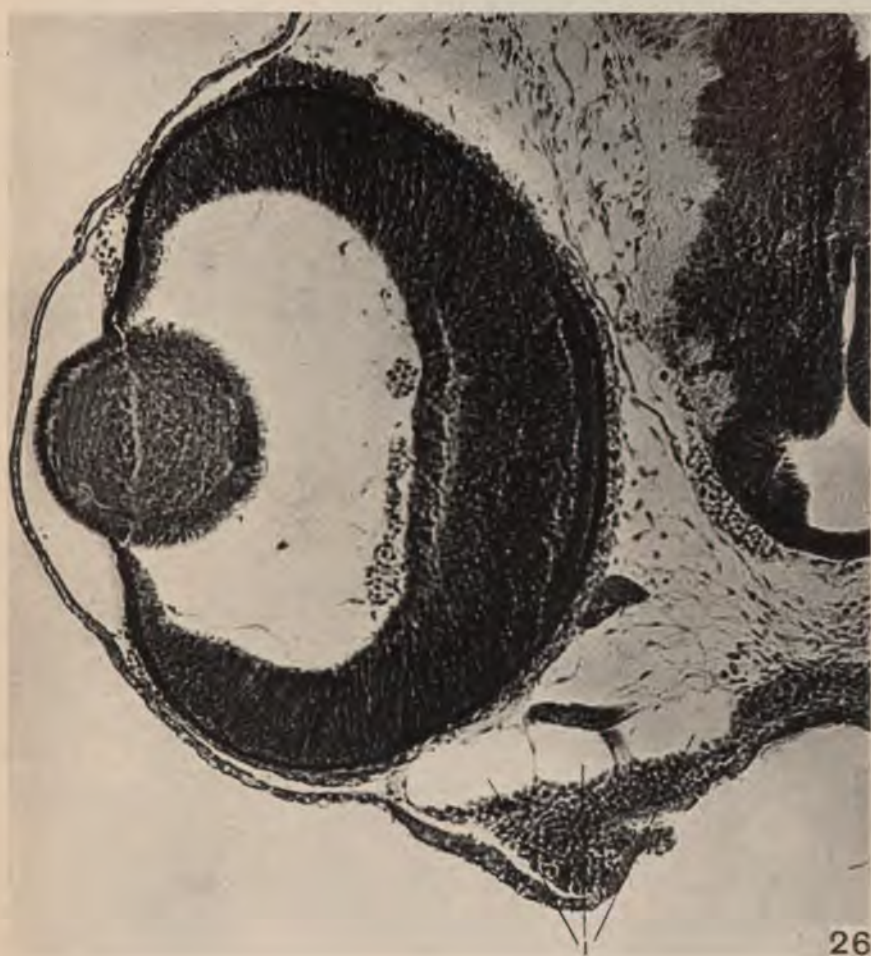


PLATE 21

EXPLANATION OF FIGURES

Fig. 27 Photomicrograph of a transverse section of a sixteen-day steelhead trout embryo, taken through an independent anlage (3') of the lateral pharyngeal lymphatic, at about the level of the first efferent aortic arches; compare 3' in figures 5 and 27. P. E. C. 751, slide, 1, section 91; injected embryo.

Fig. 28 Photomicrograph of a transverse section of a seventeen-day steelhead trout embryo, taken through independent anlagen (3') of the lateral pharyngeal lymphatic, at about the level of the first efferent aortic arches; compare 3' in figures 6, 7 and 28. P. E. C. 740, slide 1, section 103; injected embryo.

REFERENCES

- | | |
|--|--------------------------------|
| 3', Independent anlagen of the lateral pharyngeal lymphatic which lie anterior to the otic communication | 15, Hyoidean artery |
| | 16, First efferent aortic arch |
| 6, Precardinal or jugular vein | 27, Root of dorsal aorta |

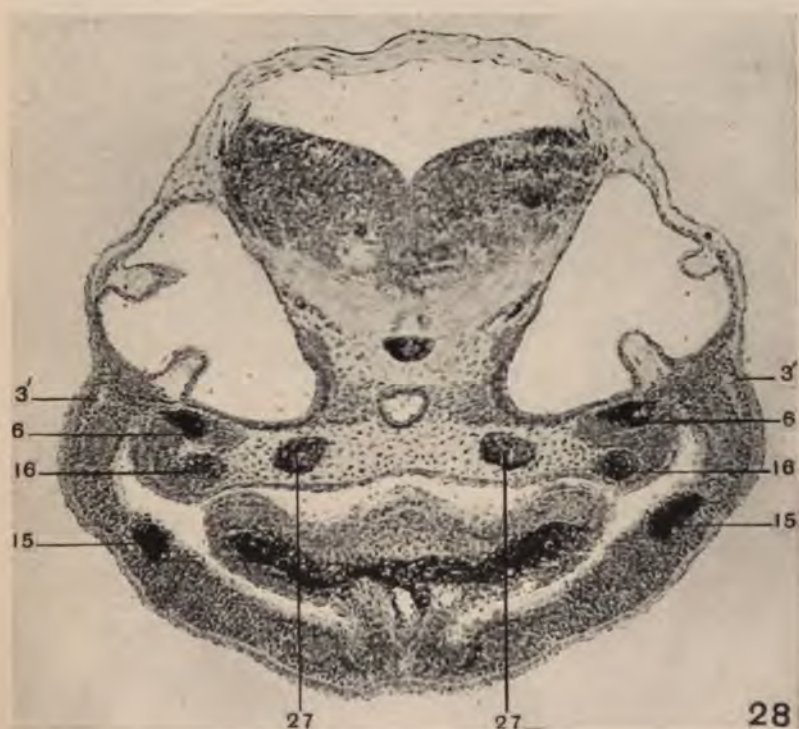
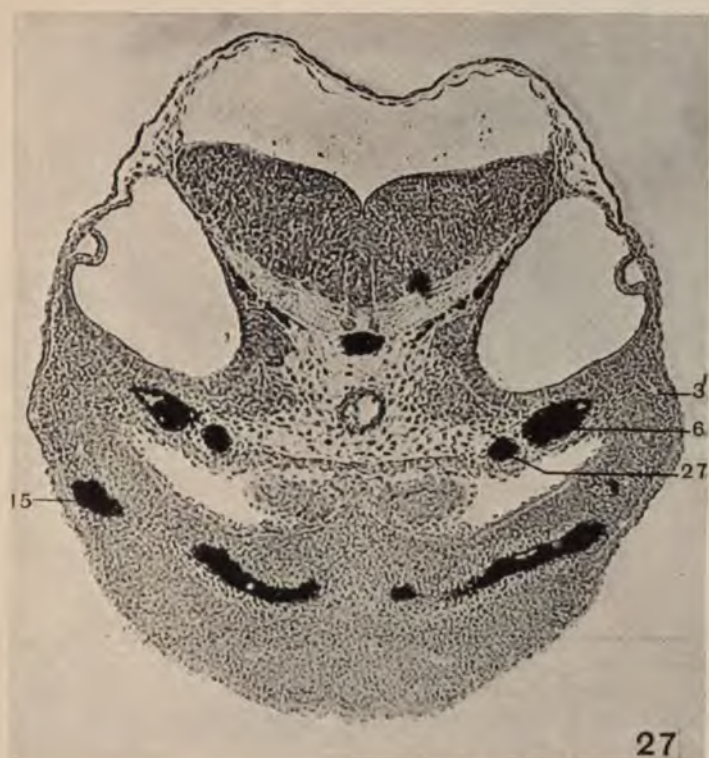


PLATE 22

EXPLANATION OF FIGURE

Fig. 29 Photomicrograph of a transverse section of a twenty-day rainbow trout embryo, taken through independent anlagen (3') of the lateral pharyngeal lymphatic, at about the level of the first efferent aortic arches; compare 3' in figures 8 and 29. P. E. C. 668, slide 1, section 122; injected embryo.

REFERENCES

- | | |
|--|--------------------------------|
| 3', Independent anlagen of the lateral pharyngeal lymphatic which lie anterior to the otic communication | 15, Hyoidean artery |
| 6, Precardinal or jugular vein | 16, First efferent aortic arch |
| | 27, Root of dorsal aorta |



PLATE 23

EXPLANATION OF FIGURES

Fig. 30 Photomicrograph of a transverse section of a twenty-two-day rainbow trout embryo, taken at about the level of the first efferent aortic arches; showing that the continuous lateral pharyngeal lymphatic (3 in figures 10 and 30) occupies the same relative position in the walls of the pharynx as its independent anlagen (3' in figures 27, 28 and 29) of earlier stages. The medial pharyngeal lymphatic (4) and independent anlagen of the precardinal lymphatics (5') are also shown in figure 30. P. E. C. 648, slide 2, section 17.

Fig. 31 Photomicrograph of a transverse section of a seventeen-day steel-head trout embryo, taken through independent anlagen (3') of the lateral pharyngeal lymphatic at about the level of the hyoidean arteries; compare 3' in figures 6 and 31. P. E. C. 740, slide 1, section 88; injected embryo.

REFERENCES

- | | |
|---|--|
| 3, The lateral pharyngeal lymphatic: a continuous vessel through which the subocular lymph sac drains into the veins in the trout | 5', Independent anlage of the precardinal lymphatic which lies contiguous to the precardinal vein and just caudal to the medial pharyngeal communication (see fig. 10) |
| 3', Independent anlagen of the lateral pharyngeal lymphatic which lie anterior to the otic communication | 6, Precardinal or jugular vein |
| 4, Medial pharyngeal lymphatic | 15, Hyoidean artery |
| | 27, Root of dorsal aorta |



PLATE 24

EXPLANATION OF FIGURE

Fig. 32 Photomicrograph of a transverse section of a twenty-day rainbow trout embryo, taken through independent anlagen (3') of the lateral pharyngeal lymphatic, at about the level of the hyoidean arteries; compare 3' in figures 8 and 32. P. E. C. 668, slide 1, section 98; injected embryo.

REFERENCES

- | | |
|--|---------------------------|
| 3', Independent anlagen of the lateral pharyngeal lymphatic which lie anterior to the otic communication | 8, Cranial end of otocyst |
| 6, Precardinal or jugular vein | 15, Hyoidean artery |
| | 27, Root of dorsal aorta |



PLATE 25

EXPLANATION OF FIGURE

Fig. 33 Photomicrograph of a transverse section of a twenty-two-day rainbow trout embryo, taken at about the level of the hyoidean arteries; showing that the continuous lateral pharyngeal lymphatic (3 in figures 10 and 33) occupies the same relative position in the walls of the pharynx, as its independent anlagen (3' in figures 31 and 32) of earlier stages. The medial pharyngeal lymphatic (4) and independent anlagen (5') of the precardinal lymphatics are also shown in figure 33. P. E. C. 648, slide 2, section 10.

REFERENCES

- | | |
|--|--|
| 3, The lateral pharyngeal lymphatic;
a continuous vessel through which
the subocular lymph sac drains into
the veins in the trout | tiguous to the precardinal vein and
just caudal to the medial pharyn-
geal communication (see fig. 10) |
| 4, Medial pharyngeal lymphatic | 6, Precardinal or jugular vein |
| 5', Independent anlage of the pre-
cardinal lymphatic which lies con- | 15, Hyoidean artery |
| | 27, Root of dorsal aorta |



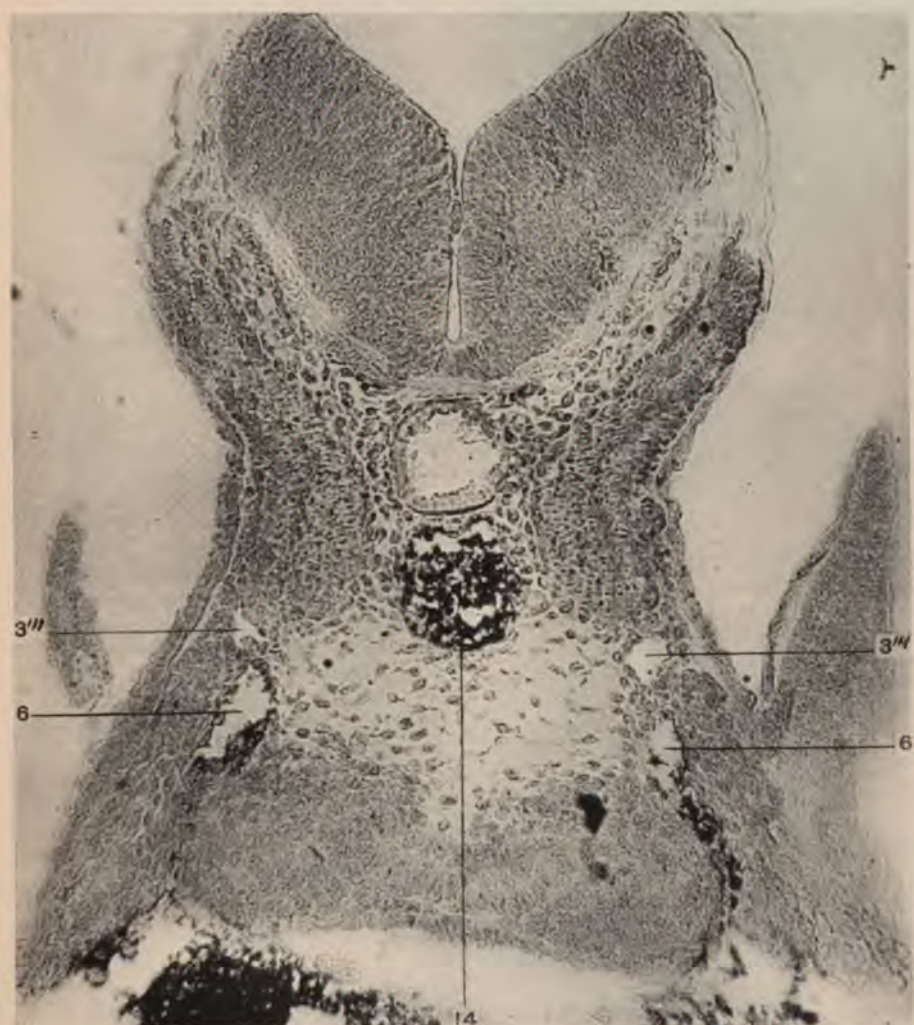
PLATE 26

EXPLANATION OF FIGURE

Fig. 34 Photomicrograph of a transverse section of a fourteen-day steelhead trout embryo, taken through the cardino-Cuvierian lymph sac (3''') found on the left side and through the more anterior of the two cardino-Cuvierian lymph sacs (3'') found on the right side of the embryo; see reconstructions of embryo, figures 1 and 2. P. E. C. 696, slide 1, section 119; injected embryo.

REFERENCES

3''', Cardino-Cuvierian lymph sac; 6, Precardinal or jugular vein
anlage of the lateral pharyngeal lymphatic which lies opposite to the cardino-Cuvierian junction 14, Dorsal aorta



34

PLATE 27

EXPLANATION OF FIGURES

Fig. 35 Photomicrograph of a transverse section of a fourteen-day steelhead trout embryo, taken through the more posterior of the two cardino-Cuvierian lymph sacs (3''') found on the right side of this embryo; see reconstruction, figure 2. P. E. C. 696; slide 1, section 122; injected embryo.

Fig. 36 Photomicrograph of a transverse section of a fifteen-day steelhead trout embryo, taken through the right otic lymph sac (3'') and an independent anlage (5'') of the precardinal (jugular) lymphatic; compare 3'' and 5'' in figures 4 and 36. P. E. C. 726, slide 1, section 116; injected embryo.

REFERENCES

- | | |
|---|---|
| 3'', Otic lymph sac; anlage of the lateral pharyngeal lymphatic which lies near the caudal end of the otocyst and which may establish a communication with the precardinal vein at the otic communication | 5'', Anlage of the precardinal lymphatic which lies contiguous to the otic communication and which establishes a connection with the lateral pharyngeal lymphatic |
| 3''', Cardino-Cuvierian lymph sac; anlage of the lateral pharyngeal lymphatic which lies opposite to the cardino-Cuvierian junction | 6, Precardinal or jugular vein |
| | 14, Dorsal aorta |
| | 17, Second efferent aortic arch |



PLATE 28

EXPLANATION OF FIGURES

Fig. 37 Photomicrograph of a transverse section of a twenty-three-day rainbow trout embryo, taken through the caudal end of the left subocular lymph sac (1); showing that the superficial facial (21) and lateral pharyngeal lymphatic communicate with the subocular lymph sac at this stage of development; compare figure 37 with figure 16. P. E. C. 968, slide 1, section 112.

Fig. 38 Photomicrograph of a transverse section of a twenty-three-day rainbow trout embryo, taken through the left subocular lymph sac (1) eleven sections anterior to the point (fig. 37) where the superficial facial lymphatic (21) communicates with the lateral pharyngeal lymphatic and the subocular sac. P. E. C. 968, slide 1, section 101.

REFERENCES

1, Subocular lymph sac

21, Superficial facial lymphatic



PLATE 29

EXPLANATION OF FIGURE

Fig. 39 Photomicrograph of a transverse section of a twenty-two-day rainbow trout embryo, taken at the level of the third and fourth efferent aortic arches (18 and 19 in figure 10); showing the relations of the precardinal lymphatics (5) to the precardinal veins (6) in the posterior region of the pharynx. P. E. C. 648, slide 2, section 51.

REFERENCES

- | | |
|--|---|
| 3, The lateral pharyngeal lymphatic;
a continuous vessel through which
the subocular lymph sac drains into
the veins in the trout | 5, Precardinal or jugular lymphatics
6, Precardinal or jugular vein
8', Caudal end of otocyst
14, Dorsal aorta |
|--|---|



PLATE 30

EXPLANATION OF FIGURE

Fig. 40 Photomicrograph of a transverse section of a 7.5 mm. embryo of *Amia* calva, taken through the independent anlage of the medial pharyngeal lymphatic (4') which does not communicate with the precardinal vein (6), nor does it connect with the latter by means of solid endothelial strand. P. E. C. 459, slide 1, section 76.

REFERENCES

- | | |
|---|--|
| 4', Independent anlage of the medial
pharyngeal lymphatic in <i>Amia</i> | 6, Precardinal or jugular vein
27, Root of dorsal aorta |
|---|--|



PLATE 31

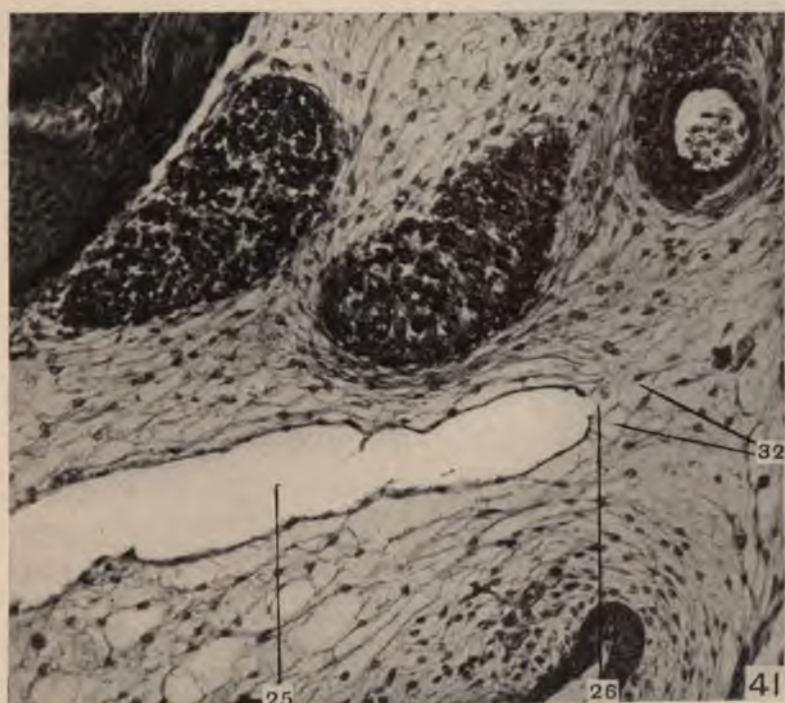
EXPLANATION OF FIGURE

Fig. 41 Photomicrograph of a transverse section of a 15 mm. embryo of *Chelydra serpentina*, taken through the periaortal lymph sinus; showing that this endothelial-lined lymph sinus (25) is in direct and open communication with a typical mesenchymal-lined tissue space (26). Columbia Embryological Collection (Col. of P. and S.) 317, slide 12, section 9.

Fig. 42 Photomicrograph of a transverse section taken through the subocular lymph sacs of a twenty-one-day rainbow trout embryo in which, as independent structures, the sacs (1) have reached the maximum stage of their development. P. E. C. 646, slide 1, section 70; injected embryo.

REFERENCES

- | | |
|---|--|
| 6, Precardinal or jugular vein | aortal lymph sinus of <i>Chelydra serpentina</i> |
| 20, Caudal end of eye | |
| 22, Carotid artery | 32, Mesenchymal tissue spaces which lie in the line of extension of the periaortal lymph sinus of <i>Chelydra serpentina</i> |
| 25, Periaortal lymph sinus of <i>Chelydra serpentina</i> | |
| 26, Mesenchymal tissue space which communicates directly with the peri- | |



MEMOIRS
OF
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
No. 5

THE DEVELOPMENT OF THE ALBINO
RAT, *MUS NORVEGICUS ALBINUS*

I. FROM THE PRONUCLEAR STAGE TO THE
STAGE OF MESODERM ANLAGE; END
OF THE FIRST TO THE END
OF THE NINTH DAY

THIRTY-TWO FIGURES

II. ABNORMAL OVA; END OF THE FIRST TO
THE END OF THE NINTH DAY

TEN FIGURES

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THE DEVELOPMENT OF THE ALBINO RAT, MUS NORVEGICUS ALBINUS

I. FROM THE PRONUCLEAR STAGE TO THE STAGE OF MESO- DERM ANLAGE; END OF THE FIRST TO THE END OF THE NINTH DAY

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INTRODUCTION

The early developmental stages of placental mammals, embracing the stages of sex cell maturation and fertilization, of segmentation, of blastodermic vesicle and germ layer formation, though subject of numerous contributions extending over many years, have in no form been completely investigated. The literature dealing with the phenomena of maturation and fer-

tilization as observed in placental mammals has in recent years been enriched by a number of studies to the extent that for certain of the mammals—bat, rabbit, guinea-pig, mouse, and rat—the data at hand are sufficiently complete to enable a clear and comprehensive presentation, based on observed facts, and permit of comparison with similar phenomena as observed in other vertebrate and invertebrate forms. As concerns the process of segmentation in placental mammals, there are still lacking sufficiently comprehensive observations embracing a number of forms to enable a clear and succinct presentation of the rate of blastomere formation, the cytomorphosis of the cells, and of the relative position of the several segmentation stages in the genital tract. This is no doubt owing to the difficulty of obtaining the necessary material timed so as to admit of proper staging, and the impossibility of making extended observations on living material. Our knowledge of the phenomena of blastoderm vesicle formation, though comprehended in its general phases, is lacking in detail, except for a very limited number of forms. The process of germ layer formation is of such fundamental importance to a clear comprehension of later developmental stages, both in phylogeny and in ontogeny, that a brief account of observed facts in any one form may not be regarded as wholly without value.

Opportunity presented itself, while stationed at The Wistar Institute of Anatomy and Biology, to collect and fix an extended series of embryological stages of the albino rat. This material has proven sufficiently comprehensive to enable a presentation of the several developmental stages of this mammal, beginning with the pronuclear stage and extending to the stage of the anlage of the mesoderm. For this period, which extends to about the tenth day after insemination, only very few of the essential stages are lacking, though for certain of the stages confirmatory preparations would have been desirable. The material at hand, however, seemed sufficiently complete to present a connected account of the stages it is hoped to cover. The embryology of allied forms, especially of the mouse, has received much more extended study than has that of the rat, though the develop-

ment of the rat has received especial consideration by Fraser, Christiani, Selenka, Duval, Robinson, Widakowich, and as concerns maturation and ovulation, by Sobotta and Burekhard, Kirkham and Burr. The pertinent literature will be considered in connection with the presentation of my own results.

MATERIAL AND METHODS

The material on which this investigation is based was obtained from albino rats (*Mus norvegicus albinus*, Donaldson)¹ taken from the extensive rat colony of The Wistar Institute of Anatomy and Biology. The experience gained in the breeding, feeding, and growth experiments, extending over many years, conducted by Donaldson and his associates and resulting in numerous excellent publications, was at my disposal while collecting this material. The material used was all carefully timed, so that sequence of stages was obtained with some degree of certainty. With care and experience, it is possible to regulate and observe insemination, so that stages may be approximated quite accurately. Kirkham and Burr state that "on several different occasions we have observed actual pairing" of the albino rat. Widakowich states that he was unable to obtain accurate data as to the age of the embryos except by observing coitus. According to this observer, a female rat permits many males to copulate in the course of several hours, receiving males 30 times or oftener, when suddenly she drives them away. Sobotta and Burekhard, on the other hand, admitted males a few hours after parturition, depending on the fact that many mammals ovulate soon after parturition. Though attempts were made, they were unable to observe pairing, and they state that the 'Dieners' charged with the care and feeding of the rat colony were only seldom able to observe attempts at pairing. At The Wistar Institute no difficulty is experienced in pairing albino

¹ Melissinos and Widakowich state having used as material the albino rat, variety *Mus rattus albinus*. Donaldson has conclusively shown, that by reason of physical characters—blood crystals, shape of the skull, etc.—the albino rat kept as pet or laboratory animal cannot be *Mus rattus albinus*, but must be *Mus norvegicus albinus*.

rats. Dr. J. M. Stotsenburg, to whose experience and careful records I am greatly indebted for the trustworthiness of the material collected, made use largely of females who had born one litter. Pairing was seldom attempted a few hours post partem, as was done by Sobotta and Burckhard, but usually about 30 days after the birth of a litter, which may have been nursed or otherwise disposed of. The great majority of females used in pairing were at the time free from 'domestic cares.' The females employed were kept in separate cages for some time before giving birth to young and until the time of mating. About 30 days after the birth of a litter, a male was placed in the cage with the female. If the female was in heat, copulation usually took place soon after. The male was left with the female for an hour to an hour and a half, during which time several pairings would occur, and at the end of which time the female would try to hide from the male, climb the side of the cage and defy him with her teeth. The male albino rat is not prostrated by the sexual act, the same male serving for several successive copulations. In case the female was not in heat, this soon became evident and the male removed, to be again placed into her cage 24 or 48 hours later. The time when the copulation was first observed was noted on the card attached to the cage and gave the time from which the age of the embryo or respective stage was reckoned. The time given is, therefore, that of 'insemination,' a term which Long and Mark have introduced to indicate "the introduction of the male sexual elements into the genital tracts of the female by the act of coitus or otherwise." This time could be accurately noted, while 'semination' which "applies to the access of the spermatozoa to the eggs in the oviducts, the coming into contact of the male and female reproductive cells" can not be accurately timed. The success attained in pairing albino rats as above stated, obviated the necessity of depending upon chance material or resorting to 'artificial insemination' as described for the mouse by Long and Mark. I am at loss to understand why Widakowich should regard the age determinations of Sobotta and Melissinos (mouse embryos) more accurate than his own, reckoned from the time

of observed coitus. The slight though observable variation in the rate of development in a series of ova of the same animal, more marked when supposedly similar stages of several animals are investigated, precludes the accurate timing of stages.

As fixing fluids, there were used Zenker's fluid, sublimate-alcohol, Flemming's fluid, Bouin's fluid, and Carnoy's fluid. After a few trials, all were discarded in favor of Carnoy's fluid, prepared by mixing 6 parts of absolute alcohol, 3 parts of chloroform, and 1 part of glacial acetic acid. This somewhat illogically compounded fluid penetrates rapidly and does not cause shrinkage. Tissues are fixed in it for several hours, then washed in several changes of absolute alcohol in which it has been my custom to store the tissues. The following procedure was practiced in all stages up to about 12 days after insemination: The animals were anaesthetized and the head severed from the body, to admit of free bleeding. The rat was then fastened to a board, and thorax and abdomen opened by a mid-sagittal incision, the abdominal walls pinned back, and the intestine elevated toward the thorax. With as little manipulation as possible, the ovaries were separated from their attachment, the mesometrium cut, the uterine horns elevated and the vagina severed. The whole genital tract was then placed on a clean slide and arranged in approximately normal position. Slight tension was maintained by tying a thread to the connective tissue removed with each ovary and bringing the threads along the reverse side of the slide and tying them to the vagina. If the slide is clean, the mesometrium of each uterine horn may be spread out evenly and caused to adhere to the slide. Ovaries, oviducts, and uterine horns may thus be spread out in normal position and each uterine horn fixed as a straight tube. When thus arranged on the slide, the preparation was placed in a relatively large quantity of Carnoy's fluid, fixed, and then transferred through several absolute alcohols. For nearly all the material used in this study, the method of fixation was as here given. In the earlier stages of material collection, attempts were made to obtain segmentation stages in warm normal salt solution. Several were thus obtained and were used to control

the observations made on sections, as will be discussed later. By cutting the oviduct at about its middle, freeing it from its mesosalpinx and cutting the uterus about 1 cm. below the insertion of the oviduct, a pipette fitted with a rubber bulb and filled with warm normal salt solution can be inserted into the uterine cavity and moderate pressure made. It is usually possible to wash into a watch crystal a certain number of the contained segmenting ova. Before reading the article by Widakowich, essentially the same method as employed by him, for isolating implanted blastodermic vesicles was developed. This may be quite readily done after fixation in Carnoy's fluid and teasing under a stereoscopic binocular. Vesicles sectioned *in situ*, however, gave on the whole more satisfactory results, so that teasing out implanted vesicles was not resorted to.

The fixed tissues were imbedded in paraffin, using xylol as a clearing fluid. For stages including those falling within the period ranging from the first to the fourth day after insemination, the ovary and oviduct to its insertion in the uterus, were embedded *en masse*. For stages falling within the period of fifth to sixth day after insemination, the uterine horns were divided into segments measuring about 1.5 cm., and sectioned parallel to the plane of the mesometrium. For later stages, after the enlargements in the uterine horns are distinctly evident, these were removed and cut severally in the three planes. The great majority of the sections were cut at a thickness of 10 μ ; certain ones at a thickness of 5 μ ; a few at a thickness of 7 μ . The sections were fixed to the slide by the water-albumen method. The great majority of the series were stained in hemalum, counterstained in Congo red. This solution, which presents certain advantages as a counterstain for embryologic tissues, is prepared as follows: 0.5 gms. of Congo red (Grübler) is placed in 100 ccm. of distilled water and the water brought to boiling. This should give a clear solution. Before cooling, add 100 ccm. of distilled water and 10 ccm. of absolute alcohol. The Congo red solution thus prepared may be kept many weeks. After staining the series in the usual way in hemalum, they are differentiated in acid alcohol, and passed through several washes of 'tap water' into distilled

water. They are then stained in the Congo red solution, which may be diluted with distilled water about five times. With the diluted solution, the counterstaining requires one to two hours. The sections are then rinsed in distilled water, differentiated in 80 per cent alcohol, dehydrated, cleared, and mounted in damar. Certain of the series were stained in Heidenhain's iron-hematoxylin and counterstained in Congo red. The drawings accompanying this contribution were nearly all drawn on coarse 'Ross board,' with the aid of the camera lucida at a magnification of 1000 diameters, using pencil and India ink. Such drawings admit of liberal reduction, and give a detail not readily obtained otherwise. Free use has been made of the Born method of reconstruction, especially for earlier stages. The majority of the models thus obtained are here reproduced.

I desire to express my sincere thanks and appreciation of the very material aid given me by Mr. Wayne J. Atwell, then Assistant in the Department of Histology and Embryology of the University of Michigan, in the making of the reconstructions of the oviducts included in this account.

OVULATION, MATURATION, AND FERTILIZATION

When this study was projected, it was the purpose to begin it with the stages of maturation and fertilization. During the time of material collection, there appeared the contribution of Sobotta and Burekhard: "Reifung und Befruchtung des Eies der Weissen Ratte," covering these stages fairly completely. Duplication of their work did not seem necessary, so that my own studies begin with the pronuclear stage, to which stage the above mentioned investigators had carried their observations. Therefore, as concerns the process of ovulation, maturation, and fertilization as observed in the albino rat, I am confined for my data to the literature; from which a brief résumé is here made.

The normal gestation period for non-lactating albino rats may be roughly estimated as from 21 to 23 days. As has been shown by King, the period of gestation of lactating albino rats varies

from a minimum of 24 days to a maximum of 34 days. The average number in a litter is six. In lactating females suckling five or less young and carrying five or less young, the period of gestation usually does not exceed 23 days and may thus be considered as normal. In lactating females suckling five or less young, while they are carrying more than five young, the period of gestation may be prolonged from one to six days. In lactating females suckling more than five young, the period of gestation is always prolonged, and may be prolonged to a maximum of 34 days. Daniel's studies on the white mouse lead him to formulate the following law: "The period of gestation in lactating mothers varies directly with the young suckled." Such exact relation between the number of young suckled and the extent of the prolongation of the gestation period was not observed by King for the albino rat.

In the albino rat, ovulation occurs spontaneously and is not dependent on copulation, which act, however, may precede or follow ovulation. Kirkham and Burr state that ovulation usually occurs about 24 hours after parturition and that the developing ova can be traced in the ovary through the two oestrus cycles preceding their discharge. Long, in his study No. 3, by Mark and Long, finds that ovulation must occur in the albino rat on an average not less than 18 hours after parturition. Sobotta and Burekhard state that ovulation always occurs within 36 hours post partem, though at very variable periods, often only a few hours after the completion of parturition; again, much later. A second ovulation period apparently occurs some 30 days post partem, as would appear from the successful pairings conducted by Dr. Stotsenburg. This agrees with the observations of Melissinos, who found that pairings were more numerous when attempted 29 days after parturition, than when attempted 20 to 21 days after parturition, as practiced by Sobotta. Semination probably takes place in the ampullar portion of the oviduct. Relatively few spermatozoa enter the oviducts and Sobotta and Burekhard estimate that the life of the spermatozoa in the genital tracts of the albino rat is only about 10 hours.

The phenomena of maturation and fertilization in the albino rat have been carefully studied by Sobotta and Burekhard, from whose account the following brief summary is taken: The behavior of the ovum of the albino rat with respect to the formation of polar bodies is very similar to that of most other mammals studied. The first polar body is given off within the ovarian follicle, the second in the oviduct and only after semination. The first maturation spindle, developed from the nucleus of the oocyte of the first order, forms usually immediately after parturition. Kirkham and Burr state "it is usually formed less than 24 hours after parturition." It is short and broad, with the chromatin scattered. The first maturation spindle lies near the center of the ovum, then passes toward the surface assuming a tangential position, and only with the beginning of metakinesis, takes a radial position. The chromosomes of the first maturation spindle, estimated as numbering 16, appear in the form of modified rings, which are divided transversely across to form short rounded rods with a longitudinal direction in the diaster stage. The first polar body is formed in the ovarian follicle and appears to be relatively large. It is evident only in the ovarian ovum, and appears to be lost soon after its formation. Its fate is doubtful. The first polar body is nearly always missing in tubal ova. Kirkham and Burr state that "the rare occurrence of the first polar body associated with the egg in the tube is to be attributed to its rapid disintegration, which begins as soon as it is formed, and may lead to complete disappearance before ovulation occurs." The second maturation division begins immediately after the completion of the first, without an intervening resting phase. The spindle formed is narrower and longer than the first, with the chromatin massed. In its monaster stage, it lies in a tangential position, with the chromatin in diads, and with the lines of division at right angles to the axis of the spindle. The appearance of the second maturation spindle in the monaster stage marks the end of the maturation phenomena in the ovary. The monaster stage of the second oocyte division was not observed in the ovary by Sobotta and Burekhard, but was seen by Kirkham and Burr. The first

division Sobotta and Burekhard regard as a reduction division, a heterotypic longitudinal division; the second as an equatorial division, a homeotypic longitudinal division. Ovulation probably occurs during the monaster stage of the second maturation division.

The tubal ova are surrounded by a relatively thin oolemma to which are adherent a variable number of discus cells. They are smaller than the ovarian ova; the latter measuring $60\ \mu$ to $65\ \mu$, the tubal ova $55\ \mu$ to $60\ \mu$. The recently discharged tubal ova are to be found in the distended ampullar portion of the oviduct, where they are found clumped together surrounded by discus cells. Semination takes place in this region. The spermatozoa usually enter while the tubal ova are in the monaster stage of the second maturation division, after which metaphase begins. The second maturation spindle assumes a radial position in the metaphase phase. The second polar body is smaller than the first, and usually lies compressed between the oolemma and the ooplasm, and is evident during fertilization and segmentation. The spermatozoan head penetrates the thin oolemma and the ooplasm; the long middle piece and tail following the head into the ooplasm, as has been shown by Coe, and Kirkham and Burr. The long middle piece, soon after penetrating the ooplasm, presents an increase in stainability, and its spiral thread becomes evident. The spiral thread, as Duesberg has shown, has its origin in the mitochondria of the spermatid. It may be, therefore, that the male sexual cell introduces mitochondria to the egg cell at the time of fertilization. Some little time after the penetration of the sperm head, this enlarges and becomes vacuolated, and diplosomes with polar rays become evident. As the sperm head begins to metamorphose, tending to the formation of the male pronucleus, the chromosome group of the dispirome of the second maturation spindle, undergoes metamorphosis to form the female pronucleus. This enlarges rapidly to form a vesicular nucleus which lies free in the ooplasm, while the metamorphosing male pronucleus, usually smaller, is accompanied by a deeply staining thread-like structure, derived from the middle piece. The centrosomes of the

first segmentation spindle are by inference derived from the sperm centrosome. The data here given, as concerns the maturation and fertilization phenomena pertaining to the albino rat, unless otherwise credited, have been drawn from the account of Sobotta and Burckhard, whose account is accompanied by excellent figures.

Long has studied in living ova of mice and rats the phenomena of maturation and fertilization. Tubal ova were placed in Ringer's solution on an especially constructed slide and spermatozoa introduced. It was possible to seminate the ova of rats with rat spermatozoa and to observe the formation of the second polar body. The formation of the second polar body, "usually near the first polar cell, may begin within five minutes to two or more hours after the spermatozoa are introduced. The constriction may be finished three-fourths of an hour later." "The first appearance is an elevation clearer than the rest of the cell. The swelling becomes higher, and at one side of the elevation there appears a depression which is the beginning of the constriction which presently encircles the whole swelling and cuts it off from the egg." Nothing could be said as to the changes which the chromatin undergoes after the spermatozoa have penetrated the egg. The eggs remained alive and apparently normal for about twelve hours, after which they began to degenerate.

PRONUCLEAR STAGE

As has been stated, my own observations on the development of the albino rat (*Mus norvegicus albinus*) begin with the pronuclear stage. The material at hand for this stage is listed in table 1, page 258.

Thus there are present in the series 34 ova showing a pronuclear stage and 9 ova showing the second maturation spindle in the monaster phase. The latter may be dismissed with the brief statement that they represent unfertilized ova. In rat No. 108, with 7 ova in the stage of the second maturation spindle, killed 24 hours after the observed copulation, there was found no trace of spermatozoa in the oviduct. Two reasons may be offered for the non-appearance of fertilization in this case:

TABLE 1

RECORD NUMBER	HOURS AFTER BEGINNING OF INSEMINATION	NUMBER OF OVA	STAGE OF DEVELOPMENT	
			Pronuclear	Second matu- ration spindle
106	24 hours	8	8	
107	24 hours	11	10	1
108	24 hours	7		7
109	24 hours, 15 min.	9	8	1
110	24 hours, 15 min.	8	8	
Total		43	34	9

Ovulation may have occurred so late that the spermatozoa may have died before the ova reached the ampullar portion of the oviduct. This explanation, it would seem, is invalidated by the fact that the position of the ova in the oviduct, as shown by graphic reconstruction, is essentially the same as in the other four rats studied, and in which fertilized ova were found, so that ovulation must have preceded the killing of the animal by some hours. The other reason, more plausible, attributes non-fertilization to a pathologic condition of the genital tract. In this rat, one ovary was distinctly pathologic, with periovarian capsule greatly distended with a sanguinous liquid, while the upper end of the uterine horn with adjacent oviduct on the other side, as seen in sections, presented evidence of inflammation and epithelial desquamation, in part occluding the lumen. It seemed evident, therefore, that the spermatozoa introduced in the genital tract were unable to penetrate to the oviduct and consummate fertilization. The other two unfertilized ova, found with ova in the pronuclear stage, were in oviducts in which no spermatozoa were found. Both in the mouse and the rat, relatively few spermatozoa reach the upper end of the oviduct; too few, it would seem, to consummate fertilization of all the ova in certain cases. In all of the ova which contained the second maturation spindle, this was in the monaster phase and in tangential position. In size, shape, and chromatin configuration, all presented the characteristics described and figured by Sobotta and Burckhard and Kirkham and Burr, therefore, need not be considered further.

The stage of pronuclei was observed in over 100 ova of the white rat by Sobotta and Burckhard. According to these observers, the two pronuclei show in the earlier stages of their development, large chromatin-like nucleoli, the number of which varies. Some little time later, one or several such chromatoid nucleolar bodies with irregularly formed chromatin masses arranged on the linin network are to be observed. At a still later time, the chromatin becomes distributed over the linin network, throughout the nuclear space, giving the appearance of a fine chromatin network. One of the pronuclei is, as a rule, somewhat smaller than the other. This is regarded as the male



Fig. 1 Tubal ova, albino rat. $\times 200$. A, rat No. 110, 24 hours, 15 min., ovum in pronuclear stage, larger nucleus female pronucleus; B, and C, rat No. 59, 2 days, 2-cell stages, thin oolemma showing in C, only partially seen in B; D, rat No. 62, 2 days, 22 hours, 3-cell stage, the nucleus of the unsegmented blastomere in the monaster phase, only one of the other two cells showing in the figure.

pronucleus, since near it the 'sperm centrum' was now and then observed. The pronuclei lie in about the center of the ovum. The pronuclear stages of my own material, observed in 34 ova, obtained 24 hours after the beginning of insemination—thus at the end of the first day of development—all present essentially the same stage of metamorphosis. As may be seen in A of figure 1, the nuclei are distinctly membraned, and are of relatively large size. The ovum here sketched measures in the stained preparation $70\ \mu$ by $62\ \mu$, and is, therefore, of slightly oval form. Sobotta and Burckhard give $55\ \mu$ to $60\ \mu$ as the size of the tubal ova, and $60\ \mu$ to $65\ \mu$ as the size of the ovarian ova in

the white rat. Kirkham and Burr give the diameter of the living unsegmented egg of the rat as of 0.079 mm. As may be seen from A and B, of figure 2, the tubal ova, even when free in the oviduct, are not of necessity spherical in shape, but often slightly compressed, as may be clearly seen in four models of tubal ova in the pronuclear stage, reconstructed at a magnification of 1000 diameters, in my possession. Depending on the plane of section, the diameter of a tubal ovum may thus vary to the extent of $5\ \mu$ to $8\ \mu$. The two nuclei in the preparation shown in A of figure 1, measure, the larger one, regarded as the female pronucleus, $23\ \mu$ by $16\ \mu$, the smaller $17\ \mu$ by $15\ \mu$. Essentially all of the chromatin is distributed over the linin network in fine granules, the larger nucleus presenting one large, faintly-



Fig. 2 Models, made after the Born method, of two tubal ova of the albino rat in the pronuclear stage. $\times 200$. A, rat No. 106, 24 hours; B, rat No. 110, 24 hours, 15 min. Reconstructions made at a magnification of 1000 diameters, figure reduced in reproduction.

staining chromatoid nucleolus. The ooplasm is finely granular, distributed so as to give the section a slightly mottled appearance. When compared with figures given by Sobotta and Burckhard (figs. 21 to 24, plates 9-10) showing pronuclear stages of the ova of the rat, my own seem to fall in about the middle of this series, thus some little time after their formation, but not immediately preceding the stage of segmentation spindle formation. In the albino rat, and perhaps in other mammals, the pronuclear stage, in its various phases of nuclear metamorphosis, must constitute a stage covering a relatively long period. If it is assumed that semination occurs about 10 to 12 hours after the beginning of insemination, such assumption being justified by the observations of Sobotta and Burckhard, according to whom the life of the spermatozoa in the genital tract of the white rat is only about 10 hours, and if it is recalled that in

living rat ova Long found that the constriction of the second polar body may be completed three-fourths of an hour after its inception, then it must be evident that the pronuclear stage extends through a period which exceeds 10 to 12 hours, since in none of my pronuclear stages obtained 24 hours after insemination was evidence of first segmentation spindle observed.

In order to determine accurately the relative position of the ova within the oviduct during the pronuclear stage and the stages of segmentation, oviducts containing ova were reconstructed after the Born wax plate method. In form, relations, and general structure, the oviduct of the albino rat is essentially the same as that of the mouse as described by Sobotta. The oviduct of the rat measures from fimbriated end to termination in the uterine horn from 2.5 cm. to about 3.0 cm. It presents eight to ten fairly constant major folds, the middle group of which is closely applied to the ovarian capsule. The upper or distal folds pierce the capsule, ending in the fimbriated end found within the capsule, while the lower or proximal folds, proximal with reference to the uterine horn, effect connection with the uterine horn. These relations are essentially the same as those described by Sobotta for the oviduct of the mouse. This observer recognizes four segments in the oviduct of the mouse, characterized by epithelial lining, nature and extent of folding of the mucosa, and thickness of the musculature. The first segment, which falls to the infundibulum, presents a thin musculature and high mucosal folds with epithelial lining consisting of relatively short cylindrical cells with distinct cuticular border and long cilia. As characteristic of this portion of the tube there are further described accessory nuclei compressed between the epithelial cells. Only this portion of the oviduct is ciliated. In the second segment, the lumen is large and the folds of the mucosa prominent. They are covered by a non-ciliated epithelium, without distinct cuticular border. The musculature is relatively thin. In the third segment the musculature is well developed with circularly and longitudinally disposed cells. The lumen is narrow and the folds are nearly absent, while the epithelium is of a simple columnar variety. The fourth segment, not so well characterized, consists of the loops which make con-

nection with the uterine horns, with folds and epithelium much as in the third segment, and a prominent musculature. In all essentials, this description applies to the oviduct of the albino rat, except that in the first segment the accessory nuclei described by Sobotta as found between the epithelial cells were not evident in the rat. In figure 3, is reproduced a model of a wax reconstruction of the right oviduct of rat No. 106, killed 24 hours after the beginning of insemination, and containing eight ova in the pronuclear stage. This oviduct measured from fimbria to termination in the uterine horn 3.2 cm. It presents 10 major folds, which folds may be recognized with more



Fig. 3 Model of right oviduct of rat No. 106, 24 hours. $\times 10$. Fimbriated end and infundibulum removed in the drawing so as to expose underlying loops; their relative position given in dotted outline. The position of the ova, which are outlined in circles, is shown as if seen through a transparent wall. The relative position of three of the eight ova found within this tube cannot be revealed in this view of the model.

or less clearness in all the models made and here reproduced. The slight difference in the relative position of these folds as seen in the several figures may be accounted for by the varying degrees of tension to which the tissues were subjected prior to fixation. In rat No. 106, the ovaries with oviduct and upper end of the uterine horn, were excised and placed in the fixing fluid without applying any tension. Of these 10 major folds, the four distal ones, those beginning with the fimbriated end, fall to segments one and two of Sobotta's designation, having a wide lumen and folded mucosa. In the figure, the position of the ova is indicated by small black circles. By reason of the relation of the folds, only five of the eight ova can be brought

to view in the aspect of the model sketched. The position of the first and the last of the series is correctly given. The ova are situated in a loop of the oviduct which is about 8 mm. from the fimbriated end. By the end of the first day after the beginning of insemination, the ova have thus travelled about one-fourth the length of the oviduct. In figure 4 is reproduced a model of a detailed reconstruction of that portion of the oviduct



Fig. 4 Model of the segment of the right oviduct of rat No. 106, 24 hours, containing the ova the general position of which is shown in Figure 3. $\times 50$. The wall is in part removed, so as to expose the lumen. Note the character of the folds of the mucosa. The relative position of the eight contained ova, all in the pronuclear stage, is clearly shown.

containing the ova, representing a loop of the tube with one side cut away, this to show the extent and character of the mucosal folds, the width of the lumen and the relative position of the several ova. The figure presents these facts so clearly that lengthy description is deemed unnecessary. The several ova are distributed through a tube segment measuring about 2.5 mm. in length. They lie free in the lumen, apparently bathed in a fluid from which there is only a small amount of precipitation at the time of fixation. Their position in the oviduct at

this stage, free in the lumen, is well shown in figure 5, which is from a longitudinal section of a loop from the left oviduct of rat No. 109, showing three ova, with but few remaining discus cells and a thread of coagulum linking the ova together, an appearance quite characteristic at this stage. The figure was drawn by aid of camera lucida from a single section. All of the ova, of which there are seven, distributed through this loop, contain two pronuclei; in none of the ova figured do the two pronuclei

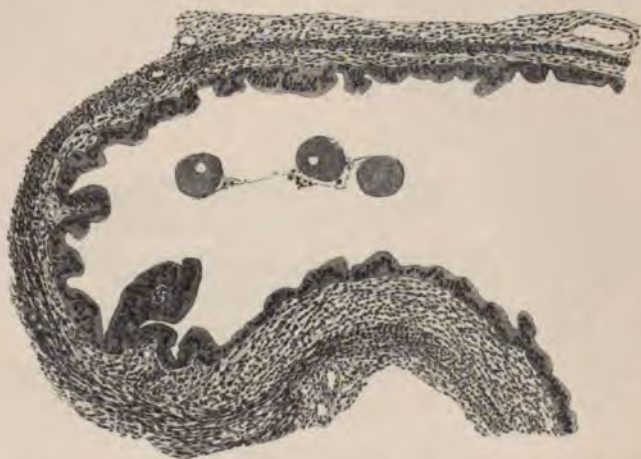


Fig. 5 Camera lucida drawing of a portion of a section of the left oviduct of rat No. 104, 24 hours, 15 min. $\times 100$. Three ova with a few discus cells, are shown as lying free within the lumen. The ova are in the pronuclear stage, not shown in this section, but readily ascertained by tracing through the series. The loop of the oviduct here shown in section is cut longitudinally, thus the folds of the mucosa are not prominent.

fall in the same section. My series contains seven oviducts with pronuclear stages, with accompanying ovary, cut serially. Only one of the oviducts, rat No. 106, was reconstructed in wax. In the other six, graphic reconstructions were made. This permits analysing the loops, determines their sequence, but does not readily admit of measuring their length. In the six oviducts graphically reconstructed, the position of the ova, the number of which varies from one to seven in the several tubes, is essentially as in the wax reconstruction figured. It would appear,

therefore, that in the albino rat, 24 hours after the beginning of insemination, the ova are to be found in the pronuclear stage, with the ova distributed in the end of the third to the beginning of the fourth major loop of the oviduct, a portion of the oviduct having a relatively wide lumen and lined by a much folded mucosa and possessing a relatively thin muscular wall, having thus migrated about one-fourth of the length of the oviduct.

SEGMENTATION STAGES

2-cell stage. The material on which my own observations of this stage are based is listed in table 2.

TABLE 2

RECORD NUMBER	HOURS AFTER BEGINNING OF INSEMINATION	NUMBER OF OVA	STAGE OF DEVELOPMENT
60	1 day, 18 hours	7	2-cell stage
59	2 days	8	2-cell stage
58	2 days, 17 hours	8	{ 7, 2-cell stages; 1, 3-cell stage
61	2 days, 18 hours	8	
62	2 days, 22 hours	11	{ 10, 2-cell stages; 1, 3-cell stage

Thus in all 40 ova after the completion of the first segmentation division and 2 ova in the 3-cell stage, in each of which the undivided blastomere presents a nucleus in mitosis.

My own material lacks stages showing the formation of the first segmentation spindle, the conjugation of the two pronuclei, and the first segmentation division. I am forced to proceed from the pronuclear stage to that showing the first two blastomeres. It was not possible to supplement my material after this was sectioned and the stages determined, since it was only after leaving The Wistar Institute that this gap in my series was recognized. This is the more to be regretted since neither Melissinos, Sobotta and Burckhard, nor Kirkham and Burr, all of whom have considered maturation and fertilization as observed in the albino rat, discuss these stages in their account. In the albino rat, the fusion of the two pronuclei on the first segmentation

spindle, and the first segmentation division would appear to fall to a period ranging from the beginning to near the middle of the second day after the beginning of insemination, probably about 30 to 32 hours after insemination. In the mouse, in which these stages have been very completely and carefully investigated by Sobotta, the conjugation of the pronuclei and the first segmentation spindle formation falls to the end of the first day after copulation. These phenomena appear to be passed through rather quickly in the mouse ovum, covering a period of only about one and a half to two hours.

The 2-cell stage with resting nuclei extends through a relatively long period. In the mouse it extends through nearly an entire day, as shown by Sobotta, who found 2-cell stages present through a period ranging from 25 hours to 48 hours after copulation. Melissinos often observed the 2-cell stage with resting nuclei in both mice and rats in material gathered 24 hours after copulation and to 44 hours thereafter. It is to be regretted that this observer does not differentiate more specifically between ova of mice and rats in his description. As a rule it is impossible to determine except by inference to which of the two varieties of ova his account refers. It may be assumed that the statements made apply equally well to the ova of either the mouse or the rat.

In my own material, the 2-cell stage was observed during a period extending from 1 day, 18 hours to 2 days, 22 hours after the beginning of insemination, thus for a period extending over more than 24 hours. In the albino rat, the first two blastomeres are equivalent cells of essentially the same size and structure, as may be seen from B and C, of figure 1, drawn respectively of ova found in the right and left oviducts of rat No. 59, killed two days after the beginning of insemination, and regarded as representative ova. The two cells of each ovum are not spherical, but of slightly oval form, with relatively large, distinctly membranated nuclei, with fine chromatin granules scattered on the linin network and a number of relatively large chromatoid nucleoli. The cytoplasm presents a granular appearance, the granules being evenly distributed throughout the cell. In my own material, I seldom find the two cells lying in the same plane,

but one cell, as a rule, rises slightly higher than the other. This is more clearly seen in reconstructions than in sections. In figure 6 are shown reconstructions of the 2-cell stages, figured in B and C of figure 1. In B, of the figures, the plane of section is at right angles to the vertical axis of the reconstruction as shown in B of figure 6, while in C of figure 1, the plane of section is parallel to the vertical axis of the reconstruction shown in A of figure 6. The equivalence or non-equivalence of the first two blastomeres of the segmenting mammalian ovum has been the subject of discussion since the time of Van Beneden's fundamental observations on the segmentation of the ovum of the rabbit. This discussion has been summarized a number of times in recent years, and need not be entered into here. Suffice to say that the consensus of opinion of the more recent contributors



Fig. 6 Models, obtained by reconstruction after the Born method, of the 2-cell stages of the albino rat. Rat No. 59, 2 days. $\times 200$.

is, that the first two blastomeres of the mammalian ovum are equivalent in size and structure if the stage is observed soon after its formation. As above stated, the 2-cell stage of the mammalian ovum extends through a relatively long period, probably about 24 hours. The two cells do not as a rule divide synchronously, the division of one preceding the other by some little time, resulting in a 3-cell stage. The cell to divide first increases slightly in size and presents a clearer protoplasm prior to its division. In a 2-cell stage, viewed in this phase of cytomorphosis, one of the cells appears slightly larger with clearer protoplasm than does the other cell, explaining the difference in size and structure observed by Van Beneden and by other observers who concur in his views. I am convinced that a difference in the size of the two cells may be accounted for by the plane of section in which they are cut, even though the nuclei of both cells are included in the section. In the figures of sections of the 2-cell

stage of the mouse, given by Sobotta and Melissinos, the nuclei of the two cells lie in about their center and essentially in the same plane. In my own material of the 2-cell stage of the albino rat it is not unusual to find the nuclei of the respective cells nearer the opposite poles of the two cells than at their centers, as shown in C, of figure 1. In B of this figure, where the two nuclei appear as lying much nearer the center of the cells, they are in reality placed much as in C, as is shown by the reconstruction.



Fig. 7 Model of the right oviduct of rat No. 59, 2 days. $\times 10$. Not quite the entire oviduct was available for reconstruction, the upper end of the uterine horn thus not shown in the figure. The position of the four 2-cell stages, each of which is outlined in a circle, found within the tube, is shown as if seen through a transparent wall.

To determine the position of the segmented ovum in the 2-cell stage in the oviduct, reconstructions were made of two oviducts. In figure 7 is shown a reconstruction of the right oviduct of rat No. 59, killed two days after the beginning of insemination. In preparing the material for embedding, this oviduct was cut not quite at its insertion into the uterine horn. The portion of the oviduct reconstructed measures 2.29 cm. Nine major loops are shown. The four ova in the 2-cell stage found in this tube are situated in the sixth to the seventh loop at a distance of about 1.4 cm. from the fimbriated end. This portion of the oviduct falls to segment three of Sobotta's designation. It is lined by non-ciliated epithelium resting on a mucosa with inconspicuous secondary folds, but presenting four or five characteristic major folds. This portion of the oviduct is closely

applied to the outside of the ovarian capsule, and conspicuous in all of the figures of models of the oviducts here presented. The detail of the distribution of the ova in the tube is given in figure 8, a reconstruction under a higher magnification of the segment of the oviduct containing the ova. The lumen is exposed so that the character of the mucosal folds may be seen. The ova are spaced in a segment of the tube measuring 3 mm., and are



Fig. 8 Model of the segment of the right oviduct of rat No. 59, 2 days, containing the four 2-cell stages as shown in figure 7. $\times 50$. Note the absence of prominent folds in the mucosa. The segment presented in the reconstruction measures 3 mm. The four 2-cell stages contained in this tube are relatively widely spaced.

in this case more widely separated than is usual for this stage. In figure 9, there is reproduced a reconstruction of the left oviduct of rat No. 62, killed 2 days, 22 hours after the beginning of insemination. This tube was also cut a little before its insertion into the uterine horn. The portion reconstructed measures 2.45 cm. In it there are found five ova in the 2-cell stage, situated about 2 cm. from the fimbriated end, and in the last loop of the third segment of the oviduct. The five ova are closely

grouped between two opposing folds of the mucosa. Their general relations are shown in figure 10, a reconstruction under higher magnification of the segment of the oviduct containing



Fig. 9 Model of the left oviduct of rat No. 62, 2 days, 22 hours. $\times 10$. Not quite the entire oviduct was available for reconstruction, thus the relative position of the upper end of the uterine horn is not shown in this figure. Fimbriated end and infundibulum removed in the drawing, so as to expose the underlying loops; their relative position is given in dotted outline. The position of five 2-cell stages, found within this tube, is given as if seen through a transparent wall.



Fig. 10 Model of the segment of the left oviduct of rat No. 62, 2 days, 22 hours, containing the five 2-cell stages, the general position of which is shown in figure 9. $\times 50$. Note the compact grouping of the ova.

the ova, cut so as to expose the lumen. At the magnification used it was not possible to reproduce in the model the exact shape of the several ova, their relative position is, however, correctly given. In all, ten oviducts, containing 40 ova in the 2-cell stage, are included in my series. Of these, two, as above given, were reconstructed by the Born method. The other eight were reconstructed graphically, beginning with the uterine

end of the tubes. In six of these, the ova are quite closely grouped as given in the reconstructions shown in figures 9 and 10. In the remaining two they were more widely spaced, about as shown in figures 7 and 8. In the oviducts taken from rats Nos. 58, 61, 62, killed respectively 2 days, 17 hours, 2 days, 18 hours, and 2 days, 22 hours, after insemination, the ova are found in a portion of the tube which corresponds very closely to that shown in the reconstruction presented in figure 9. In rat No. 60, killed 1 day, 18 hours after insemination, the ova are more widely spaced and are situated in a segment of the oviduct approximately one loop nearer the fimbriated end than that given in figure 7, a model of the oviduct of rat No. 59, killed two days after insemination.

In one of the segmented ova of rat No. 60, the two blastomeres resulting from the first segmentation division are distinctly separated by a space equal to about one-half of the diameter of each of the cells. No oolemma is discernible. The two separated cells appear normal in size, shape, and structure, as do also their nuclei. They lie free in a slightly distended portion of the lumen, and appear not to have been separated as a consequence of manipulation. The possibility of each developing separately is suggested, and may be offered as a possible explanation of the occurrence of very small embryos now and then found among others showing normal development. King states that "On dissecting pregnant females (rats) one frequently finds one or more embryos that are much smaller than the rest. While in some instances such small embryos appear normal and are presumably either runts or embryos that have resulted from superfecundation, in the majority of cases they are pathological, probably because of faulty implantation of the ovum." My own material contains pathologic ova and embryos in different stages of development. This portion of the material will be considered in Part II, where the possibility of the occurrence of half embryos will be discussed.

As may have been seen, the 2-cell stage of the albino rat covers a period of somewhat more than 24 hours, extending from about the middle of the second day until toward the end of the third

day after the beginning of insemination. During this period the segmented ova migrate in the oviduct for a distance equaling nearly half its length. The trustworthiness of the material, it would seem to me, is shown by the fact that in the shorter time stages the segmented ova are situated nearer the fimbriated end, while in the longer time stages they approach the region of the insertion of the oviduct into the uterine horn. This is clearly shown in the reconstructions shown in figures 7 and 8.

A 3-cell stage was observed only twice: in one of eight ova contained in the oviducts of rat No. 58 (2 days, 17 hours) and in one of eleven ova found in the oviducts of rat No. 62 (2 days,



Fig. 11 Two views of each of three models of 4-cell stages of the albino rat. Rat No. 50, 3 days, 1 hour. $\times 200$. A, B, and C, gives a side view, A', B', and C' a vertical view, of each of the three models.

22 hours). All the other ova found in these two animals were in the 2-cell stage. In the two 3-cell stages noted, the undivided blastomeres of each ovum presented a nucleus in mitosis; in one, in the monaster phase, in one, in the diaster phase. The division of the first two blastomeres, resulting in the 4-cell stage, it would appear, occurs in the albino rat toward the end of the third day. The material gathered at the beginning of the fourth day after insemination presents throughout a 4-cell stage. In D of figure 1 is shown reproduced one of the sections of a series of six sections including one of the ova in the 3-cell stage. Only one of the two cells resulting from the division of one of the first two blastomeres is included in the section; the cell in mitosis represents the undivided blastomere.

4-cell stage. The material includes the oviducts of two rats, Nos. 50 and 63, killed 3 days and 1 hour after the beginning of insemination, with twelve ova in the 4-cell stage. In figure 11, there are shown two views of each of the models obtained by reconstruction after the Born method, at a magnification of 1000, of the three 4-cell stages found in the oviducts of rat No. 50. The drawing of the reconstructions do not present the conventional figures of the 4-cell stage of the mammalian egg. In none of the twelve ova of this stage was the plane of section such as to include all of the four cells in one section. Nearly all



Fig. 12 Cross-section of right oviduct of rat No. 50, 3 days, 1 hour. $\times 100$. This section contains two cells of a 4-cell stage of the albino rat, slightly compressed between the folds of the tubal mucosa.

lie in a portion of the tube which presents a relatively narrow lumen, and appear as if slightly compressed between the folds of the mucosa. I am not disposed to regard this as a resultant of fixation, due to contraction at the time of fixation. In figure 12 is reproduced a cross section of the right oviduct of rat No. 50, passing through a 4-cell stage. It is evident that in shape the two cells included in the section, conform in the main to the form of the lumen, the mucosa appearing as slightly retracted to one side of the egg mass. This conformity in shape of cell mass to the form of the lumen I find quite general in my material showing segmentation stages of the albino rat, to some extent

even in the 2-cell stage, more clearly shown in the 4-cell and later segmentation stages, as will appear from further reconstructions presented. It would seem to me reasonable to assume that these cell masses are of such plasticity that they are molded by the tubal mucosa rather than they would compress the mucosa and maintain an inherent form. A number of segmented ova in presumably the 6- and 8-cell stages were removed from oviducts by injection and studied in warm normal salt solution, in a living state. In the warm normal salt solution the morula masses



Fig. 13 Model of right oviduct of rat No. 50, 3 days, 1 hour. $\times 10$. A short segment of the upper end of the uterine horn, lower part of the figure, is included. The fimbriated end and a part of the infundibulum removed in the drawing so as to expose the underlying loops; their relative position is indicated in dotted outline. The position of the four ova in the 4-cell stage, at the beginning of the last loop of the oviduct, is shown as if seen through a transparent wall.

presented a nearly spherical form, conforming to the conventional illustrations of the same. In none of the sections of fixed material of my series was this the case. The form of the cell mass, assumed by the segmenting mammalian ovum in early stages of segmentation, therefore, seems to me a question more for academic discussion than one of fundamental importance. The right oviduct of rat No. 50 (3 days, 1 hour) was reconstructed after the Born method. This model is reproduced in figure 13, and includes the uppermost end of the uterine horn. The oviduct

measures 2.8 cm. and contains four ova in the 4-cell stage, situated at the beginning of the last loop leading to the uterine horn, 2.25 cm. from the fimbriated end, thus in the fourth segment of the oviduct as of Sobotta's designation. In figure 14 is reproduced a detailed reconstruction of the segment of the oviduct containing the ova, with the convex portion of the wall of this loop, as shown in figure 13, removed. The section reproduced in figure 12, passes through the lower of the three upper ova, shown in reconstruction in figure 14. In the figure of the reconstruction as also in that of the section, is shown the groove in which these three ova lie. The other oviducts con-



Fig. 14 Model of the segment of the right oviduct, rat No. 50, 3 days, 1 hour, containing the four ova in the 4-cell stage, the general position of which is shown in figure 13. The convex portion of the wall of the loop containing the ova is removed, so as to expose the lumen.

taining 4-cell stages were reconstructed graphically, beginning with the uterine end. The position of the ova in each is essentially as given in the model reproduced in figure 13.

8-cell stage. In rat No. 57, killed 3 days, 17 hours after the beginning of insemination, there are found in the left oviduct, six segmented ova in the 8-cell stage and one segmented ovum in the 11-cell stage. The right ovary and oviduct was injured in the process of embedding and could not be used for sectioning. The ova are spaced in the loop of the oviduct which terminates in the uterine horn. Six of the segmented ova were reconstructed, the seventh was not detected at the time the reconstructions were made. The six models obtained are reproduced

in figure 15, two views of each model being shown. Five of the models, A to E, show 8-cell stages. In F, there is figured an 11-cell stage, three of the cells having completed the next following division. As may be seen from the figures, the form of these morula masses is not spherical but in the main slightly oval, with further irregularities better shown in the models than in the illustrations, due to the fact that the egg masses conform to the shape of the lumen of the oviduct in the region in which they are found. The mucosa lining the segment of this



Fig. 15 Models, obtained by reconstruction after the Born method, of 8-cell and 11-cell stages of the albino rat. Rat No. 57, 3 days, 17 hours. $\times 200$. Two views of each model is presented. A-A', to E, E' are of models of 8-cell stages; F and F' of a model of a 11-cell stage.

oviduct containing the ova presents four quite regular longitudinal folds. In figure 16, there is presented a model of a detailed reconstruction of the segment of the oviduct containing the ova, their relative position in the tube and their relation to the major folds is clearly shown. One of these folds it was necessary to in part remove so as to bring to view in the drawing certain of the ova. In figure 17, there is reproduced a portion of one of the sections of the series from which the model shown in figure 16 was made. The fold of the mucosa occupying the center



Fig. 16 Model of the segment of the oviduct, rat No. 57, 3 days, 17 hours, containing the ova shown in fig. 15. $\times 50$. A portion of the wall of the oviduct and a part of the major folds of the mucosa are removed in the drawing so as to expose the contained ova. The relative position of the seven ova found in the tube is shown, as also the extent and character of the folds of the mucosa. The exact form of each of the several ova could not be reproduced in the model at the magnification used; their position is given correctly.



Fig. 17 Camera lucida drawing of a portion of a section of the left oviduct of rat No. 57, 3 days, 17 hours. $\times 100$. This section is of the series of sections from which the models shown in figures 15 and 16 were made. Sections of four 8-cell stages, as seen in a single section, are included. The close proximity of three of these ova, their relation to the tubal wall and mucosal folds is to be noted.

of the drawing, and greatly occluding the lumen, is the fold removed in the model. In this very fortunate section four of the morula masses are included; all are of the 8-cell stage and represent in section the four ova which are placed closely together as seen in the model figured in figure 16. In figure 19, A, there is reproduced at higher magnification another of the sections of the series, including the right one of the three ova in close apposi-



Fig. 18 Model of the left oviduct of rat No. 51, 4 days. $\times 10$. A short segment of the upper end of the uterine horn was included in the reconstruction, lower end of the figure. The position of three of the morula masses, 12-cell to 16-cell stages, in the terminal part of the oviduct is to be noted, a further one is located in the upper part of the uterine horn. These are shown as if seen through a transparent wall. A fifth morula, situated in the uterine horn about 1.5 cm. from the entrance of the oviduct, is not included in the figure.

tion as seen in figure 17, showing six of the eight cells, each cut in the plane of its nucleus. In both of these figures (figs. 17 and 19) the morula masses, as seen in the sections drawn, present a quite regular oval outline. In succeeding sections, in which the mucosal fold and the wall of the oviduct approximate, the cross diameter of each of the four morula masses becomes greatly reduced, they appearing in the final sections of the series in which they are included as narrow, non-nucleated bands of protoplasm.

This series, it seems to me, corroborates the statement previously made, that the detail of form of the living segmenting ova of certain mammals, while in transit through the oviduct, is in a great measure dependent on the configuration presented by the lumen of the oviduct in the particular region in which they are found.

12-cell to 16-cell stages. Rat No. 51, killed 4 days after the beginning of insemination, presents the end of the segmentation stages in the oviduct. In the genital tract of this animal there were found eight morula masses, five on the left side and three on



Fig. 19 Sections of morula stages of the albino rat. $\times 200$. A, 8-cell stage, rat No. 57, 3 days, 17 hours; six of the eight cells, each cut in the plane of its nucleus, are included in the section figured. B, C, and D, 12-cell to 16-cell stages, from right oviduct, rat No. 51, 4 days.

the right side. It is somewhat difficult to determine definitely the number of cells constituting each of the morula. The number appears to vary between 12 and 18, though nearly all of the morula masses show certain nuclei in mitosis. The left oviduct with a short adjoining segment of the uterine horn was reconstructed. Slight tension was applied to the tissue prior to fixation, which accounts for the elongation of the proximal loop of the oviduct. The model is reproduced in figure 18. As is evident on study of this figure, three of the morula masses are situated in a portion of the oviduct just prior to its insertion in the uterine tube. These are closely grouped between folds of the mucosa. A fourth morula is found in the uppermost part of the uterine

cavity, just distal to the opening of the oviduct, lying free in a slightly distended portion of the lumen. This morula is of irregular discoidal form, presenting an appearance which suggests that it was fixed soon after it escaped from the oviduct. A fifth morula, of regular oval form, comprising very probably 18 cells, all of which present resting nuclei, is lodged in a shallow pit of the uterine mucosa a little over 1 cm. from the tubal opening. This portion of the uterine horn was not included in the reconstruction, the position of this morula is not, therefore, indicated in the figure. It is evident that this tube was fixed while the several morula masses were in transit from the oviduct to the uterine horn, which occurs, to judge from the material at my disposal, at the end of the fourth day after the beginning of insemination. The morula masses of the right tube are situated in the oviduct just before its point of insertion into the uterine horn, in about the same relative position as are the three upper morula masses of the left side, as shown in the reconstruction. They are of discoidal form, in close relation and appear to comprise, the one 12, the other two 14 to 16 cells. In B, C and D of figure 19 are reproduced sections of each of these three morula stages. The figures, however, are delusive in that the section for each passes through the greatest diameters of the respective morula.

The material at hand permits the conclusion that in the albino rat the segmenting ova pass from the oviduct to the uterine horn at the end of the fourth day after the beginning of insemination, probably in the 12-cell to 16-cell stages. With the beginning of the fifth day, as will appear from further discussion, all of the ova are to be found in the uterine horn.

SUMMARY OF SEGMENTATION STAGES, RATE, AND VOLUME CHANGES

The following summary of the data (table 3) gained by a study of the models of oviducts containing ova in stages from the pronuclear to 12-cell to 16-cell stages in which latter stage transit to the uterine horn occurs, is presented to indicate rate of transit within the oviduct. The regularity of the rate of transit

as revealed in the summary may perhaps speak for the trustworthiness of the age data as concerns my material.

It will be observed that the ova approach the uterine end of the oviduct while in the 2-cell stage; transit through the last portion of the oviduct, where the greater part of the segmentation occurs, being relatively slow. It is hoped that these data, for the accuracy of which I am dependent on reconstructions, may be of service to others who may desire to collect segmentation stages of the albino rat.

TABLE 3

RECORD NUMBER	SIDE RECONSTRUCTED	AGE	NUMBER OF OVA	STAGE	LENGTH OF OVIDUCT	DISTANCE OF OVA FROM FIMBRIA	RELATIVE LENGTH OF TUBE TRAVERSED
					cm.	cm.	
106	R	1 day	8	pronuclear	3.2	0.8	0.25
59	R	2 days	4	2-cell	2.29*	1.4	0.61
62	L	2 days, 22 hrs.	5	2-cell	2.45*	2.0	0.82
50	R	3 days, 1 hr.	4	4-cell	2.8	2.5	0.90
51	L	4 days	5	12- to 16-cell	2.86	2.86	1.00

*Not the entire length of oviduct was available for reconstruction.

In order to obtain the volume changes of the ova during transit through the oviduct, beginning with the pronuclear to 8-cell to 11-cell stages, the following procedure was adopted. As has been shown by my figures, reconstructions were made at a magnification of 1000 diameters of ova presenting the stage in question. The sections of my series measure 10 μ in thickness. In order, therefore, to obtain the correct third dimension, it was necessary to use wax plates 10 mm. thick, in actual practice, five superimposed 2 mm. plates. For the majority of the sections of my series this procedure was relatively simple. However, there was usually a question as to the thickness to be ascribed to the first and last section of any given series, since it was evident, both from the appearance of the section, as seen under the magnification used, and the appearance of the model, that the end sections did not measure 10 μ in thickness, and it

was necessary to reduce proportionately the thickness of the wax plate representing them. As a rule, these were made about one-half the thickness of the other plates. The irregularities revealed by the rough model after superimposing the respective plates, not so marked as might be supposed considering the thickness of the plates used, were adjusted, not by trimming the model and cutting away wax, but by smoothing with warm irons. The possibility of error is admitted, but since all of the models were made in the same way, errors if committed were probably essentially the same for all of the models. The volumes of the models were obtained by weighing the water displaced by each, and after making the necessary temperature corrections, reducing weight of water displaced to volume. The average of several determinations is given in table 4.

TABLE 4

RECORD NUMBER	AGE	STAGE	ACTUAL VOL. OF EGG MASS IN C. MM.	AVERAGE VOL. PER STAGE GIVEN IN C. MM.
106	1 day	pronuclear	0.00015058	0.000155693
106	1 day	pronuclear	0.00014317	
106	1 day	pronuclear	0.00015775	
106	1 day	pronuclear	0.00017127	
59	2 days	2-cell stage	0.00016240	0.000172565
59	2 days	2-cell stage	0.00018273	
50	3 days, 1 hr.	4-cell stage	0.00018338	0.000162443
50	3 days, 1 hr.	4-cell stage	0.00015520	
57	3 days, 17 hrs.	8-cell stage	0.00018893	
57	3 days, 17 hrs.	8-cell stage	0.00016040	
57	3 days, 17 hrs.	8-cell stage	0.00018653	0.000183516
57	3 days, 17 hrs.	8-cell stage	0.00018193	
57	3 days, 17 hrs.	8-cell stage	0.00019979	
57	3 days, 17 hrs.	11-cell stage	0.00021025	

The uniformity of the figures giving the actual volume of the egg mass, as determined by the weight of the water displaced by the models of the respective ova reconstructed, leads me to feel that the errors committed in reconstruction were not serious. The last column of the table, giving averages, is of interest since it shows a very slight increase in the volume of the egg mass during segmentation and transit through the oviduct. Following the pronuclear stage, which, as has been seen, ex-

tends through a relatively long period and into the beginning of the second day, by which time the ova have migrated about one-fourth of the length of the oviduct, there occur only three successive mitotic divisions, including the first segmentation division, namely mitoses resulting in 2-cell, 4-cell and 8-cell stages while the ova are in transit in the oviduct. In making this statement it is assumed that in the successive segmentations, the several cells divide synchronously, which is not in conformity with the fact. These three mitotic divisions are spaced at intervals of about 18 hours. In the next following division, the fourth, the ovum passes from the oviduct to the uterine horn. Since the normal gestation period of the non-lactating albino rat is only 21 to 23 days, this slow rate of increase in volume and multiplication of cells during the first four days of development is of especial interest and is very probably to be accounted for by the inadequacy of the food supply of the ovum during its transit through the oviduct.

The presence or absence of the oolemma has not been considered in discussing the segmentation stages of the albino rat. In my own material, the oolemma was clearly observed in certain of the 2-cell stages, but not in the 4-cell nor 8-cell stages. Widakowich reports that he has observed in the albino rat, loss of the oolemma even in the 2-cell stage. Since all of the material covering these stages was fixed in Carnoy's fluid, a fluid with a relatively large glacial acetic acid content, it may be questioned as to whether the fixative used may not be in part responsible for the early disappearance of the oolemma, though neither Hubrecht nor Sobotta considers the presence or absence of an acid in the conserving fluid of special moment in the fixation of the oolemma. Sobotta finds that the oolemma disappears in the ova of mice during the 8-cell stage. The early disappearance of the oolemma in the albino rat may be offered as an explanation of the fact that the egg mass during segmentation and transit through the oviduct does not, as a rule, present a spherical form but appears compressed and molded to fit the form of the lumen. A similar explanation is offered by Sobotta to account for the irregularity of form assumed by the ovum of the mouse after loss of the oolemma. In the forms in which the oolemma

persists through the later stages of segmentation, as for instance in the rabbit, the morula mass presents a spherical form. The transit of the ova through the oviducts is effected, very probably, through peristaltic action of the muscular coat, since only a relatively short portion is lined by ciliated epithelium. Whether or not there exists a rhythmic periodicity in the peristaltic action, it is impossible to state. The fairly regular rate of transit argues for the presence of some regulatory mechanism. The compact grouping often presented by a series of ova in transit through the oviduct, especially after reaching the portion with narrower lumen, suggests peristaltic action.

The literature dealing with the segmentation stages of the albino rat is very meagre. Grosser figures what is presumably an 8-cell stage. His figure 27 is referred to only incidentally in the text, but in the accompanying legend it is stated that the figure shows "three ova of the white rat in process of segmentation, with zona pellucida, in transit through oviduct, three and one-half days after insemination." If I am right in interpreting these ova as in the 8-cell stage, this corresponds very closely to my own observation on rat No. 57, 3 days, 17 hours (figs. 15-17). It is impossible to draw definite conclusions as to the segmentation of the ova of rats from the account of Melissinos. This observer while he states that his material includes the ova of mice and rats, and while considering segmentation mentions the ova of both forms, discusses them without differentiating between the two. His figures all refer to ova of the mouse. Selenka, Robinson, and Widakowich, who have contributed to our knowledge of the embryology of the albino rat, do not include the segmentation stages, to be found in the oviduct, in their account.

The rate of segmentation and the time of transit through the oviduct, as given in the literature for certain other mammals is as follows: Sobotta has shown for the mouse that the 2-cell stage is reached about 24 hours after copulation, the ovum remaining in this stage to about the 48th hour. The 4-cell stage was observed at about 50 hours, the 8-cell stage at 60 hours, and the 16-cell stage at 72 hours 'post coitum.' The ova of the mouse pass into the uterine horn about 80 hours post coitum,

thus the beginning of the fourth day, in a stage in which 16 cells up to 32 cells may be enumerated; the oolemma having been lost in the 8-cell stage. The data furnished by Melissinos as concerns the mouse, are as follows: The 2-cell stage is obtained at the end of 24 hours after copulation, the 6-cell stage during the first 12 hours of the second day, and the 28-cell stage during the second 12 hours of the second day. The ovum is said to pass into the uterine horn at the end of the third day after copulation, retaining its oolemma. The account of Sobotta seems the more reliable. Hensen describes a 2-cell stage in the guinea-pig 22 to 24 hours after copulation, and Bischoff records that the ovum of the guinea-pig passes into the uterine horn while in the 8-cell to 16-cell stage, toward the end of the third day. Heape, who has described very fully the segmentation stages of the mole (*Talpa europea*) gives no data as to the rate of segmentation. In the explanation of the figures presented it may be noted that the ova figured, showing 2-cell to 15-cell stages, were taken from the oviduct. His figure 20, showing an ovum 'fully segmented' was obtained from the anterior end of the uterus. Assheton gives for the rabbit the following data: The 2-cell stage is obtained about 24 hours and the 4-cell stage about 26 hours after coitus. The third series of divisions begins about 28 hours after coitus, so that by the end of the second day a typical morula of 16 cells to 20 cells is to be found. Between 73 hours and 96 hours the beginning of the blastodermic vesicle formation is to be noted. Ova obtained 80 hours after coitus, still surrounded by the oolemma, were removed from the uterine horn. Data as to the relative position of the ova in the oviduct in the several stages of development discussed, are given. As concerns the sheep, Assheton states that the ova pass into the uterine horn early on the third day after mating. The pronuclear stage is to be observed the second day, and the first segmentation at the end of the second day. By the fourth day, with the ova in the 8-cell stage, they are found in the upper end of the uterine horn. The blastodermic vesicle formation begins with the 16-cell stage. Again, according to Assheton, the ova of the pig pass to the uterus about the third day after fertilization, if I read him rightly, reaching the uterus in the 4-cell stage, although ova in the 2-cell

and 3-cell stages were obtained from the upper end of the uterine horn. The presence of 2-cell stages in the uterine horn has also been noted by Keibel, in *Erinaceus europaeus*, by Van Beneden in the bat, and by Hubrecht in the insectivor *Tupaya javanica*. Finally, it may be noted that according to the observations of Bischoff, the segmenting ovum of the dog occupies 8 to 10 days after insemination in transit through the oviduct.

COMPLETION OF SEGMENTATION AND BLASTODERMIC VESICLE FORMATION

The material covering the end stages of segmentation and the early stages of blastodermic vesicle formation is listed in table 5.

TABLE 5

RECORD NUMBER	AGE	NUMBER OF OVA	STAGE
64	4 days, 14 hrs.	5	Early stage of blastodermic vesicle formation
52	4 days, 15 hrs.	8	Morula, beginning of segmentation cavity, early stage of blastodermic vesicle
55	4 days, 16 hrs.	1	Early stage of blastodermic vesicle
68	4 days, 16 hrs.	4	Early stage of blastodermic vesicle
53	5 days	7	Early stage of blastodermic vesicle
56	5 days	5	Early stage of blastodermic vesicle

Thus there are at hand 30 ova, showing late morula stages, the beginning of segmentation cavity formation and early stages of the blastodermic vesicle, falling in the latter half of the fifth day after the beginning of insemination. In all of the uteri from which this material was taken, the ova are spaced in the uterine horns about as in later stages of development; they lie free in the uterine lumen, are in the main ovoid in form, their long axis presenting no definite relation to the long axis of the uterine horn. In preparing this material for sectioning, it was the custom to cut an entire uterine horn into segments measuring about 1.0 cm. to 1.5 cm. in length. These segments were then embedded so as to admit cutting longitudinally and in a plane parallel to the plane of the mesometrium. Cut in this way, the majority of the ova were cut longitudinally or nearly so, others in an oblique plane, others again, crosswise. Since it

is impossible to orient the ova prior to sectioning, the securing of desirable sections is a matter of chance. The difficulty is further enhanced by reason of the fact that owing to shrinkage as a result of the action of the fixing fluid, the ova in the vesicle stage are apt to be more or less folded, so that even though the plane of section may be that desired, the resultant sections lose in value by reason of this folding.

It has been shown that in the albino rat, the ova pass from the oviduct to the uterine horn toward the end of the fourth day. During the first half of the fifth day, the migration of the ova from the oviduct to the uterine horn appears to be completed, so that by the second half of the fifth day the ova are spaced in the uterine horn about as after fixation to the uterine mucosa. As to the factor or factors which play a rôle in the descent of the ova through the uterine horn and their fairly regular spacing, my own material gives no data; these changes occurring, apparently, during the first half of the fifth day, covering which my material is lacking. Widakowich, who has given especial study to these questions, presents the following considerations: In the downward migration of the ova in the uterine horn, it cannot be assumed that the ova are capable of active movement nor can their motion be ascribed to the action of gravity. While peristaltic action may play a part, it is difficult to see how peristalsis could be so regulated as to space the ova fairly regularly within the uterine cavity. The presence of a ciliated epithelium in the human uterine cavity during the intermenstrual period suggested the presence of a ciliated epithelium in the uterine horn of the rat. After many preparations had been searched in vain for its presence, Widakowich found short cilia, not more than $2\ \mu$ long in the epithelium lining the uterine cavity of a rat killed four days after copulation, and containing ova in the blastodermic vesicle stage. It would appear, therefore, that the uterine epithelium of the rat presents a ciliary border for only a relatively short time, and that the transportation of the ova within the uterus is effected by the cilia. Mandl also found, his material however not including the rat, that cilia are present in many animals on the epithelium lining the uterus only at certain periods, and perhaps only relatively

short periods. While the presence of cilia may explain the migration of the ova in the uterine tube, Widakowich can offer no conclusions as concerns the regulatory mechanism by means of which the ova are spaced at fairly regular intervals in the lumen of the uterus. In none of my sections of the uteri of albino rats, obtained during the fifth day after insemination, have I been able to note the presence of cilia on the uterine epithelium, even when sections were studied under the oil immersion. After reading the account of Widakowich, their presence was looked for in all pertinent stages, but without success. Especially in rat No. 50, in which the ova were passing from the oviduct to the uterine horn was careful search made, but nothing like a distinct ciliary border, composed even of short cilia, was ascertained. In the left genital tract of this rat, as has been stated, three ova were found in the terminal part of the uterine end of the oviduct, one in the uterine lumen just distal to the mouth of the oviduct, and one a little over a centimeter from this opening. The latter was lodged in a shallow depression of the uterine mucosa, as is characteristic for stages lying free in the lumen.* The question as to whether this ovum was permanently lodged is difficult to answer. If this is assumed, it is further necessary to assume that the other ova would need to pass it to reach the more distal parts of the uterine lumen.

The literature contains no definite statements as concerns the reactions of the epithelium and mucosa of the uterus to the ova soon after their appearance in the uterine cavity. Widakowich summarizes the views by stating that "It is generally stated, that so long as the ova lie free, the uterus shows no changes." He himself notes that at this time the mucosa presents evidence of marked new formation of capillaries. Burckhard, who had at his disposal a large number of stages showing implantation of the ovum of the mouse, discusses at length the appearance presented by the uterus soon after the ova enter the same and the lodgment of the ova therein. This observer notes that in the non-gravid uterus of the mouse, the lumen lies more or less eccentric, and towards the mesometrial border.

The lumen is not smooth, but presents numerous radially arranged folds, certain of which are relatively deep. Essentially the same characteristics pertain to the mucosa of the uterus, soon after the beginning of gravidity. As the ova pass from the oviduct to the lumen of the uterus they become lodged in certain of the mucosal folds, and generally in certain of the deeper ones to be found along the anti-mesometrial border. I find Burckhard's account of the form of the lumen of the uterine horn, of the structure of the mucosa in early stages of gravidity, and the lodgment of the ova, pertaining to the mouse, applies equally well to the albino rat. No reason can as yet be given as to why the ova are lodged in the mucosal folds in which they are found, and not in others. So far as may be ascertained from the sections, the particular mucosal folds in which the ova are found, do not differ in form and structure from neighboring folds. It is possible that by reconstruction of the epithelial lining of the entire uterine horn in pertinent stages, certain characteristics of form and position might be revealed as possessed by certain mucosal folds which make them especially favorable for the lodgment of the descending ova. Such reconstructions, however, have not been made. Burckhard states that in the mouse, about the middle of the fifth day, after the ova have been in the uterine cavity for a number of hours, there may be observed the first changes in the uterine wall. The changes consist primarily in a flattening of the uterine epithelium. In the immediate region where implantation is to occur, the lining epithelial cells present instead of a cylindric form, a cubic form. The area is sharply demarked from the surrounding epithelium, the transition of cubic to cylindric epithelium being marked by a sharp-lipped epithelial ledge. In my own material of the rat covering these stages, the uterine mucosa likewise presents shallow pits, in the immediate regions where the ova are lodged, lined by slightly flattened, cubic epithelium, very much as described by Burckhard for the mouse. Widakowich presents an excellent figure (fig. 2 of his contribution, rat four days after fertilization—'Befruchtung') showing clearly the relations of the ova to the uterine wall. In this rat, the

uterine epithelium presented a ciliary border, present even in the shallow pit lodging the ovum sketched. He argues from this that the shallow depression and the flattening of the epithelium are not a result of pressure exerted by the vesicle, as thought by Sobotta and Melissinos, but must be due to an active change in the epithelium itself. The mucosa underlying the shallow pits presents at this stage no change of structure. I am thus in accord with Widakowich when he states that he was not able to observe in the mucosa of the rat in the early stages of gravidity, the giant cells described by Disse as found in the uterine mucosa of *Arvicola arvilis*, in similar stages.

The form presented by the ova of the albino rat, in the late morula stages and the early stages of blastodermic vesicle, is ovoid, as may be seen from the figures to be presented. Widakowich is inclined to believe that the form of the blastodermic vesicle of the rat is in a measure dependent on the general form of the space in which it is lodged. He figures two vesicles (figs. 1-2) one of which is nearly spherical, the other of distinctly oval form. Duval (figs. 73-83) presents vesicles having ovoid, triangular, and spherical forms. Christiani's figures covering these stages, are too schematic to be of any value in drawing conclusions. I fear Robinson's account is based on imperfectly fixed material. He states that "toward the end of the fifth day, or the commencement of the sixth day, the longitudinal axis of the blastodermic vesicle is 125 μ long. During the sixth day, that axis is diminished, first to 95 μ , and then to 64 μ , after which it again increases, and at the commencement of the seventh day, it is 121 μ ." Neither Fraser nor Selenka describes nor figures the stages here considered. In the mouse, according to the accounts of Melissinos, Burekhard, and Sobotta, the form of the blastodermic vesicle in early stages is spherical.

The more specific consideration of my own material I shall introduce with a discussion of three stages taken from the uterus of rat No. 52, killed 4 days, 15 hours after the beginning of insemination. In A, of figure 20, there is reproduced the middle one of seven sections of a late morula stage. This morula is of ovoid form, measuring 85 μ in its long diameter, 54 μ in its

broad diameter—that is, in plane of sections, and since it passes through seven sections of 10 thickness, measures approximately $70\ \mu$ in its third dimension. It consists of 24 cells, as estimated by counting the nuclei contained in its several sections. The cells vary in size as well as in shape. The nuclei are for the main of spherical form, presenting one or several large nucleoli and fine chromatin granules. This morula is found within a fold of the mucosa, each side of which presents a slight depression, lined by slightly flattened epithelium. This morula mass lies



Fig. 20 Sections of morula mass and early stages of blastodermic vesicle of the albino rat. $\times 200$. A, B, C, rat No. 52, 4 days and 15 hours. D and E, rat No. 68, 4 days and 16 hours. A, late stage of morula; B, shows the very beginning of the formation of the segmentation cavity; C, D, E, early stages of blastodermic vesicle, in E, a distinct covering layer in the thicker portion or floor of the vesicle is evident.

free in the lumen of the mucosal fold, and not in contact with the uterine epithelium. The outline and extent of the shallow depressions found in the opposing walls of the mucosal folds conform to shape and size of the morula mass contained, which appear as if slightly retracted as a result of fixation.

In B, figure 20, is figured one of the sections of a series passing through a morula stage, comprising as estimated 30 cells and measuring $90\ \mu$, by $60\ \mu$, by approximately $50\ \mu$, in which the very beginning of the formation of the segmentation cavity is shown. Near one pole the outermost cells have separated slightly from the more deeply placed cells, so that an irregularly shaped

cavity, eccentrically placed and passing clearly through two of a series of five sections of $10\ \mu$ thickness, is evident. The small cleftlike cavity is bounded by the surrounding cells, the outline of which is distinct. So far as may be judged from the appearance noted as presented in the two sections in which this cavity is found, this arose as a single space and as a result of the separation of the enclosing cells.

In C, of figure 20, there is presented a slightly older stage showing the blastodermic vesicle formation and measuring $80\ \mu$, by $50\ \mu$, by approximately $50\ \mu$, comprising as is estimated, 34 to 36 cells. Unfortunately, the lower part of this vesicle is slightly folded as is shown in the lower left of the figure. The appearances presented in the sections are reproduced as faithfully as could be. Owing to the folding, a portion of the thin wall is cut tangentially. The more darkly colored curved line represents in reality the outer boundary of this portion of the vesicle. The segmentation cavity in this vesicle is distinctly larger than that shown in B of this figure. In the section reproduced the segmentation cavity is bounded for the greater part by four somewhat flattened cells, the increase in the size of the cavity being accompanied, it would seem, by a flattening of the enclosing cells.

In these three closely approximated stages, which, since they are taken from the same uterus are probably separated in time of development by only short intervals, the cells though varying in size and shape, show no essential or fundamental difference in structure, neither in cytoplasm nor nuclei; nor do they show any regularity in arrangement. Only few mitotic figures are to be observed; none in the morula mass shown in A, and but two in each of the other two stages, shown in B and C. Judging from these preparations, one would be led to conclude that segmentation cavity formation in the albino rat is not associated nor accompanied by active cell proliferation. This point will be referred to again after the presentation of further material at hand. In slightly older stages of the blastodermic vesicle than here considered, the thicker portion of the vesicle is designated by Sobotta and others as its floor, which is directed

toward the mesometrial border of the uterine horn, while its thinner portion is designated as its roof, directed toward the antimesometrial border. Therefore, in slightly older stages than thus far figured, the vesicle lies with its long axis approximately at right angle to the long axis of the uterine horn. In further description of the blastodermic vesicle, I shall use the term 'floor' and 'roof' as here specified. In D and E of figure 20, there are reproduced typical sections of the two blastodermic vesicles taken from the uterus of rat No. 68, killed 4 days, 16 hours after insemination. Vesicle D measures $90\ \mu$ by $30\ \mu$ by approximately $60\ \mu$, and is of distinctly ovoid form and slightly compressed. This vesicle is found lying free in a long but narrow fold of the mucosa, both sides of which are slightly molded in conformity with the form of the vesicle. The long axis of the vesicle is still parallel to the long axis of the uterine horn. The roof of the vesicle appears as if slightly contracted, though when traced through the series of six sections it does not appear folded. The roof is composed of only a few cells, perhaps seven in all. The segmentation cavity presents a regular outline. This vesicle supports the contention of Widakowich, that the form of the blastodermic vesicle of the rat is dependent in a measure on the form of the space in which it is found. Vesicle E, of figure 20, measuring $85\ \mu$ by $45\ \mu$ by approximately $40\ \mu$, presents a roof that is slightly folded and shows an early stage in segmentation cavity formation. A figure of the vesicle is included since it represents more clearly than any other blastodermic vesicle of the albino rat in my possession, a differentiation of a layer of surface cells in the mass constituting its floor. This is a question to be more fully considered in further discussion.

In all the measurements of blastodermic vesicles thus far given, even in those given for the morula mass shown in A, figure 20, it is evident that one of the short diameters is appreciably shorter than the other. The vesicles are not only of ovoid form, but slightly flattened, so that even when not folded, the form of the vesicle as seen in section, even when cut parallel to the long axis of the respective vesicles, is dependent in a measure on the plane of the section, whether parallel to the longer or the shorter

of the two cross diameters. This may be seen from the series of drawings made of a blastodermic vesicle cut cross-wise, taken from the uterus of rat No. 68, from which were also taken the two vesicles shown in D and E of figure 20. This series of figures is shown in figure 21, in which are reproduced in serial order the seven successive cross sections into which the vesicle was cut. It measures $65\ \mu$ by $38\ \mu$ by approximately $70\ \mu$, and is found at the bottom of a mucosal fold, found at the mesometrial border, and is resting with one side on the epithelial lining of a shallow pit, the other wall of this mucosal fold, also showing a shallow pit, is slightly retracted. From a study of this series



Fig. 21 A complete series of cross-sections of an early stage of blastodermic vesicle of the albino rat. $\times 200$. Rat No. 68, 4 days and 16 hours. A to C, sections through roof of vesicle, showing segmentation cavity; D to G, sections through floor of vesicle.

of sections, I feel certain that the plane of section is cross and not oblique to the long axis of the vesicle. The roof of this vesicle passes through three sections, A, B and C. The segmentation cavity has thus a depth of less than $30\ \mu$. The overlapping of the cells surrounding the segmentation cavity is to be noted, especially as seen in B of this figure. This arrangement of the cells may explain how the cavity may be enlarged without a material increase in the number of the enclosing cells—in part, by a flattening out of the cells, in part by a rearrangement of the relations of the cells. In the figures of the sections passing through the floor of this vesicle, D to G, attention is drawn to the size, form and relations of the cells and to the fact that there is no distinct covering layer. In this series of sections, there are

shown in all 35 nuclei, two of which are in a late diaster phase. By excluding the nuclei that appear to be cut in two, appearing thus in two successive sections, I estimate that the blastodermic vesicle is made up of only about 30 to 32 cells.

In figure 22, there are reproduced typical sections of four blastodermic vesicles taken from the uterus of rat No. 53, killed five days after the beginning of insemination. This uterus contained in all, eight vesicles, one of which was distinctly pathologic. The four vesicles selected for reproduction and discussion present each certain characteristics worthy of consideration. Vesicle A, which presents an early stage of segmentation cavity forma-



Fig. 22 Sections of early stages of blastodermic vesicle of the albino rat, $\times 200$. Rat No. 53, 5 days.

tion is of interest owing to the number of mitotic divisions it contains. As a rule, I have noticed only a few mitoses at this stage. In this vesicle, which measures $90\ \mu$ by $55\ \mu$ by approximately $40\ \mu$, there are no less than five mitoses to be noted, three of which are in cells forming the roof of the small segmentation cavity, and are included in the section figured. This is the only vesicle in my possession in which an increase in the size of the segmentation cavity is accompanied by active mitoses in the cells bounding it. The vesicle lies free in the uterine lumen, one wall of which is only slightly pitted. In B of figure 22 is reproduced a section of a blastodermic vesicle, measuring $90\ \mu$ by $55\ \mu$ by approximately $45\ \mu$. It is evident that this vesicle

passes through five sections of $10\ \mu$ thickness, though one of the end sections, the fifth section of the series, seems to have fallen out during manipulations of staining and mounting, since the preceding, or fourth section does not quite complete the series. This vesicle lies free in the lumen of the uterus, and there is evident only a shallow pit in the mucosa juxtaposed. In this vesicle the cells forming the roof of the segmentation cavity are relatively numerous, and are not markedly flattened, and in one an early mitotic phase is recognized. Here again cell proliferation appears to have accompanied increase in size of segmentation cavity.

The vesicle shown in C of figure 22, measuring $130\ \mu$ by $30\ \mu$ by approximately $40\ \mu$, lies free in a long, narrow fold of the uterine mucosa, in close proximity to a shallow mucosal pit, lined by cubic epithelium; the pit conforming in shape and extent to the form of the side of the vesicle presented to it. Therefore, it would seem that the form of the vesicle as seen in sections of the fixed material is essentially the same as that obtained *in vivo*. The two vesicles, typical sections of which are shown in B and C of this figure, are almost in identically the same phase of development, although their form as seen in sections differs markedly. The plasticity of the living blastodermic vesicles is no doubt such that their form is in a great measure dependent on the shape of the mucosal fold in which they are lodged. In D of figure 22, there is reproduced a section of a blastodermic vesicle which points to a stage of development which is slightly more advanced than that shown in previous figures. The vesicle measures $100\ \mu$ by $70\ \mu$ by approximately $50\ \mu$. The roof enclosing the segmentation cavity is slightly folded; a portion of its wall is thus cut tangentially, as shown in the lower left of the figure. The segmentation cavity is distinctly larger than that shown in the preceding figures, and is bounded by a relatively large number of cells, fourteen in that portion of the roof sketched in this figure, one of which is in a mitotic phase. The mass of cells constituting the floor appears as slightly compressed, in consequence of a slight intravesicular pressure which aided in the enlarging of the segmentation cavity.

The cells forming the roof of the segmentation cavity do not appear so distinctly flattened as is the case in certain of the vesicles figured in figures 20 and 21. It would appear, therefore, that at least two factors are operative in the increase of size of the segmentation cavity after its anlage—a flattening out and consequent increase of the exposed surfaces of the enclosing cells, and secondly, a cell proliferation; and it would appear that both of these factors may be operative from the time of the beginning of segmentation cavity formation.

Early stages in the blastodermic vesicle formation in the albino rat have been previously described by Robinson, Christiani, Duval, and Widakowich; Selenka's youngest stage is slightly older than any discussed by me. My own observations are wholly in accord with those of Widakowich in so far as his account covers early stages of blastodermic vesicle formation. He discusses and figures, however, only two vesicles, obtained four days after fertilization—'Befruchtung,' in each of which the segmentation cavity presents a smooth and regular outline and is of appreciable size. The observations of the other observers who have considered these stages will be discussed in connection with a very brief presentation of much more comprehensive observations on the mouse in similar stages of development. Of these latter, those of Sobotta ('03) are based on abundant and apparently well fixed material. Sobotta begins his discussion with the consideration of three ova taken from the same mouse, the second half of the fourth day after fertilization, each of which shows beginning of segmentation cavity formation, one of which was cut in longitudinal axis and is figured in his figure 1. This ovum is interpreted as showing that the segmentation cavity arises not as a single space, but as a number of disconnected spaces, which later become confluent and form a single space. A similar observation was made by Van Beneden on the bat, a fact which Sobotta uses to support his contention that the mouse ova studied by him were of normal structure. Melissinos gives a number of figures showing early stages in the formation of the segmentation cavity in the mouse. His figures 21 and 22 (66 hours) are not unlike my own figures shown in B of

figure 20 and A of figure 22. According to this observer, the segmentation cavity arises as a single space, due to an accumulation of fluid secreted by the cells of the morula. This secretion is evidenced by globule-like droplets which are shown in his drawings as adhering to certain of the cells bounding the segmentation cavity. In my own preparations of the albino rat, I find no evidence which would lead to the supposition that the segmentation cavity does not begin as a single space nor do I find any evidence of secretory globules as described by Melissinos. Selenka has described quite fully two blastodermic vesicles of the mouse, lying free in the uterine lumen. His account of their structure, supported by two somewhat diagrammatic figures, is as follows: The wall of the vesicle is formed by a layer of covering cells—'Deckzellen'—constituting a covering layer—"Deckschicht or Rauber's layer." The space enclosed by this layer, for about one-third to one-half of its extent, contains the 'formative cells,' for the remainder it contains fluid. The covering cells and formative cells are said to be separated by a sharp line. The formative cells are in all parts separable into two fundamental germ layers. An inner layer, bordering the cavity and constituting the entoderm, is said to be composed of cells possessing more deeply staining nuclei, irregular outline, with tongue-like processes which extend into the cavity, and a granular protoplasm; further, of cells which are more clear, more peripherally placed, and which constitute the ectoderm. Each of these fundamental germ layers consists of a single layer of irregularly formed cells. According to the observations of Selenka, therefore, the floor of the vesicle consists of three layers of cells; an outer covering layer—"Deckschicht" or 'Rauber's layer'—an inner layer of entodermal cells, and an intermediate layer of ectodermal cells. Jenkinson's account reads as follows: "There are present (1) an outer layer, one cell deep, of trophoblast, which is continuous over (2) an inner mass which becomes differentiated into the embryonic epiblast and the hypoblast, and which is quite distinct from the overlying trophoblast, as my specimens invariably show." In Jenkinson's figures 1 and 2, giving early stages of the blastodermic vesicle, there is not shown a differentiation of the inner

mass into ectodermal and entodermal cells; the outer layer, covering layer, Rauber's cells, or trophoblast, is clearly differentiated from the inner mass by a distinct space. Duval has recognized in early stages of blastodermic vesicle formation of the mouse and rat, in the thicker part of the vesicle, entodermal and ectodermal cells. The former are of irregular form, possess granular protoplasm and are said to possess the property of ameboid movement. The remaining cells are recognized as ectoderm; a distinct covering layer is not recognized. In Christiani's figures (rat), which are, however, so diagrammatic as to be of little value and are evidently drawn from poorly fixed material, entodermal cells, ectodermal cells, and covering cells may be recognized as per legends. Melissinos (mouse), while not describing definitely a peripheral or covering layer, states that outer cells of the thicker pole, like the cells enclosing the segmentation cavity, stain less deeply than do the more centrally placed cells. In earlier stages of vesicle formation, neither in figures nor in text as given by this observer, do I find reference to a differentiation into ectodermal and entodermal cells. The observations of Selenka, Duval, Robinson, Jenkinson, and others, bearing on the structure of the blastodermic vesicle of the mouse and the rat in early stages of development have been so thoroughly and critically reviewed by Sobotta that an extended discussion has here been deemed unnecessary. It may here suffice to say that while Sobotta's observations were made and his discussions based on ova obtained from the mouse, my own observations made on the albino rat are in agreement with his and support the contention that in early stages of blastodermic vesicle formation a differentiation of the thicker part or the floor of the vesicle into a covering, Rauber's cell, or trophoblast layer, and a further differentiation into ectodermal and entodermal cells, is not to be made: we differ in our accounts of the beginning of the formation of the segmentation cavity. An outer or covering layer is suggested in certain of my own preparations, most clearly in that sketched in E of figure 20. However, a uniform difference in structure and reaction to staining reagents of the outer layer of cells is not present in my own preparations. None of my own preparations gives evidence of

such an early differentiation of ectoderm and entoderm as given by Selenka, Duval, Christiani, and others. Cells of irregular outline with tongue-like projections, such as figured by Selenka and Duval I have not observed. The cells constituting the floor or the thick part of the vesicle all present essentially the same structure, while the segmentation cavity, as soon as it presents appreciable size, shows a smooth and regular outline. In figures 1 and 2, of Widakowich's communication, excellent figures of early stages of blastodermic vesicles of the albino rat, there is presented no evidence of a trophoblast layer nor a differentiation of ectodermal and entodermal cells. My own figures, 20 to 22, were drawn with the aid of camera lucida at a magnification of 1000 diameters and with the use of an intense Welsbach light. They are reduced five times in reproduction. With the exceptions of cell outlines, which as sketched do not in the preparations fall in the same optical plane, and are sketched more sharply than is perhaps warranted, the figures portray quite accurately the structural appearances presented, so far as may be with the use of a single color.

BLASTODERMIC VESICLE, BLASTOCYST, OR GERMINAL VESICLE

The material on hand is listed in table 6.

TABLE 6

RECORD NUMBER	AGE	NUMBER OF VESICLES
75	5 days, 15 hours	6
91	5 days, 16 hours	2
88	5 days, 21 hours	7
89	5 days, 21 hours	6
73	6 days	10
74	6 days	5
99	6 days	6
100	6 days	10
104	6 days	6 Total 58

During the sixth day, the blastodermic vesicle of the albino rat increases in size relatively rapidly. The greater portion of its wall is, at this stage, composed of a single layer of flattened cells. The vesicles are not as yet attached to the uterine wall, though the uter-

ine mucosa shows a distinct reaction to their presence. Localized thickenings of the uterine mucosa, sufficient to cause localized swellings of the uterine tube, indicating the position of the ova, are evident. I have experienced more difficulty in successfully fixing the vesicles during this stage than any of the earlier or later stages studied. Although my material contains 58 vesicles of the stage under consideration, none of them may be regarded as being well fixed, and the majority of them are so folded as a result of contraction during fixation that they are of little value as objects for especial study. That the vesicles are still unattached to the uterine wall is readily determined by the fact that the shrivelled vesicles are found lying free in the depressions of the uterine mucosa, lined by a low cubic epithelium, intact throughout, and retaining its normal relation to the mucosa. The molding in these mucosal depressions no doubt gives the size of the respective vesicles as *in vivo*.

It is not my purpose at this time to consider more than superficially the changes affecting the uterine mucosa during ovum implantation in the albino rat. It is hoped that this may be the subject of a future communication. It is the purpose in the present communication to confine consideration to the development of the ovum itself. Many of the observations recorded by Burckhard on the implantation of the ovum of the mouse and the formation of the decidua, I find equally adapted to similar phenomena in the albino rat. Differences are to be observed in certain details which it is not the purpose to enter into here. Grosser gives a number of excellent figures (67 to 70, and 112 to 116) showing implantation and decidua formation in the albino rat; to these the interested reader is referred for the present. The thickening of the mucosa affects primarily its antimesometrial portion. During this process of thickening, the mucosal fold in which the ovum primarily finds lodgment, becomes deepened and converted into a funnel shaped crypt communicating with the uterine lumen, and surrounded by the 'Eibuckel,' or oval fold. Burckhard's schematic figures (text figures 2 to 4) may be consulted to make the phenomenon intelligible.

In figure 23, there are reproduced representative sections of five blastodermic vesicles falling to the end of the sixth day after insemination. None of these five vesicles can be regarded as well fixed. All show a certain amount of distortion, much more evident were the entire series of each of the respective vesicles shown. The form of the blastodermic vesicle of the albino rat at this stage of development, as indicated by the molding of the uterine mucosa, is ellipsoid. Their size as *in vivo*, when distended and of regular outline, again as indicated by the molding of the uterine mucosa, is slightly larger than would be



Fig. 23 Sections of blastodermic vesicles or blastocysts of the albino rat. $\times 200$. A and C, rat No. 99, 6 days; B, D, E, rat No. 100, 6 days. *y.ent.*, yolk entoderm; *p.ent.*, parietal layer of entoderm; *p.ect.*, parietal or transitory ectoderm.

supposed from the drawings presented. By reason of this distortion, exact measurements of size cannot be given.

In A of figure 23, there is reproduced that portion of one of the sections of a blastocyst (rat No. 99, 6 days) which passes through its floor; the thin roof of this vesicle was so folded that its inclusion in the drawing was deemed undesirable. However, its floor or the germinal disc, seems to have retained its normal form and structure, presenting when traced through the series a regular concavo-convex, discoidal form. It consists in the main of three layers of cells of polyhedral type; toward the border of the disc, of two layers of somewhat flattened cells, the peripheral layer being continuous with the single layer of cells

forming the roof of the vesicle, not shown in the figure, and known as the parietal or transitory ectoderm. In the floor or germ disc, there is evident a single layer of cells bordering the segmentation cavity or blastocele and possessing a more granular protoplasm, which stains a little more intensely in Congo red. Their differentiation and characteristic reaction to staining agents is at this stage of development not quite so distinct as in slightly older stages. This layer of cells, similar to that described by Sobotta for the blastodermic vesicle of the mouse in essentially the same stage of development, he has termed the yolk entoderm, 'Dotter entoderm,' a designation which is here followed. In the more superficial layer or layers of cells no characteristic differentiation is observed. In no portion of the floor of this vesicle was a distinct covering or trophoblast layer recognized.

In the vesicle, a section of which is reproduced in B of this figure (rat No. 100, 6 days), the floor or germ disc presents essentially the same structure as that shown in A. The vesicle shown under B, was also folded, especially its roof, which was drawn to one side and was thus not cut through its entire length in the section figured. Furthermore, the section chosen for drawing does not pass quite through the center of the germ disc, but a little nearer to one of its edges, which probably accounts for the fact that there is recognized for the greater part only a single layer of cells, superimposed over the yolk entoderm, which layer is continuous with the parietal or transitory ectoderm forming the roof of the vesicle. The cells forming the yolk entoderm constitute a single layer and are quite distinctly differentiated; one of the cells shows a mitotic phase. The roof of the vesicle formed by the parietal or transitory ectoderm, is composed of a single layer of flattened cells with flattened nuclei, the form and structure of which is more correctly shown in the right half of the roof wall, which in the section is cut transversely, while the left half, owing to the folding, is shown as cut obliquely.

In C of figure 23 (rat No. 99, 6 days), there is shown a greatly compressed blastodermic vesicle, taken from a series of cross sections of the uterine horn. In this figure there is reproduced the fifth of a series of 10 sections of 10 μ thickness; therefore,

the third dimension of the vesicle is approximately $100\ \mu$. It is evident that had this vesicle been cut in a favorable plane at right angles to the present series, or parallel to the mesometrial plane, its form would have approached that of a circle. I have in my possession one vesicle of this stage of development, similarly compressed, cut parallel to the plane of compression, in which almost the entire roof falls within a single section of $10\ \mu$ thickness. The structure of the vesicle shown in C is very similar to that shown in A and B of this figure. The normal form of this vesicle is quite readily reconstructed from a study of the series of sections into which it has been cut. The cells of the yolk entoderm are evident. The parietal or transitory ectoderm constituting the roof consists of a single layer of much flattened cells, with relatively few nuclei, having, as seen in cross section, a long ovoid form, which, when seen in surface view present a regular, nearly circular outline (see lowermost nucleus in the figure). In similarly compressed vesicles cut parallel to the plane of compression, the germ disc may appear as consisting of three to four layers of cells. In an imaginary section passing in a plane at right angles to that figured in C, and having perhaps a slight obliquity, the germ disc would appear as if much thicker than that shown in A and B of this figure. Such sections may readily lead to false conclusions.

It seems evident from a study of the material at my disposal that during the sixth day after the beginning of insemination in the albino rat, the blastodermic vesicle or blastocyst, which has its anlage in the latter part of the fifth day, enlarges relatively rapidly; this largely owing to a distension of the segmentation cavity or blastocele. This enlargement is accompanied by a flattening and extension of the enclosing roof cells and by a rearrangement of the cells of the floor, which is reduced in thickness to a discoidal area, the germinal disc or germ area, forming about one-fifth to one-sixth of the wall of the vesicle and consisting of two or three layers of cells. During the rearrangement of the cells which constitute the floor of the vesicle, those adjacent to the segmentation cavity or blastocele differentiate to form the anlage of the yolk entoderm. The remaining cells of the ger-

minal disc, having all essentially the same structure, are of irregular polyhedral form and are mutually compressed. To designate them as a distinct germ layer at this stage seems inappropriate. A differentiation into a layer of covering cells and a layer of formative ectoderm (Selenka) is not to be made. Active cell proliferation as evidenced by mitotic figures does not appear to accompany this enlargement of the vesicle. This phenomenon seems rather to be accomplished by a rearrangement of the cells constituting its floor, however, primarily by an extension and consequent flattening of the cells forming the roof of the vesicle. A similar stage is shown for the mouse by Sobotta ('03) in his figures 3, 4, and perhaps 5, of mouse vesicles from the fifth day after fertilization—'Befruchtung'. Sobotta had at his disposal much more perfectly fixed vesicles than my material contains. The structure of these vesicles as given by this observer, both as depicted in figures and text, is very similar to the presentation given by me. He also recognizes in this stage the anlage of the yolk entoderm. Figure 30, accompanying the account of Melissinos (mouse, 84 hours) presents a similar stage, although he figures fairly distinctly a layer of covering cells, which if I read him correctly, however, is of only transitory existence. None of the figures given by Robinson and Jenkinson is comparable with figures A, B, C, of figure 23 of this account.

In D, of figure 23 (rat No. 100, 6 days) there is reproduced a section of a blastodermic vesicle which on superficial study presents a somewhat later stage of development than those shown in A to C, of this figure. It is, however, only very slightly older than the three vesicles discussed. Vesicle D, cut in good longitudinal direction, is in reality much more folded than appears from the section figured. Its floor or germ disc is compressed in a plane parallel to that of the plane of section, so that the germinal disc is cut obliquely and not transversely, and thus appears thicker in the section than it in reality is. A distinct layer of covering cells, continuous with the cells of the parietal ectoderm, is evident. Such a layer of covering cells is figured by Selenka, Jenkinson, and Duval. The yolk entoderm has differentiated and extends by perhaps three cells, in the

section figured, onto the layer of parietal ectoderm. Selenka and Duval, who regard the cells of the primary entoderm as having ameboid properties, are disposed to regard the entodermal cells found lining the parietal ectoderm as having wandered from their seat of origin to the side wall of the vesicle. Sobotta sees no evidence of such wandering of the primary or yolk entodermal cells, but suggests that they are drawn to their position on the wall of the vesicle during its increase in size; their wandering, therefore, is more relative than absolute. Certain cells nearer the edge of the yolk entoderm, having attachment to the parietal ectoderm, which attachment they retain as the vesicle enlarges, are thought to be drawn from their close relation to the yolk entoderm and to appear as scattered cells lining the parietal ectoderm. Now and then, such cells may divide, resulting in further distribution. Sobotta's suggestion seems to me to be more in accord with the observed facts. In vesicle D, the roof, consisting of a single layer of flattened, parietal ectodermal cells, presents several major folds as well as minor folds. The latter particularly account for the variation in thickness of the wall of the vesicle as seen in sections. At the lower left of the figure is seen a portion of the wall as seen cut on the flat, the shape of the two nuclei here shown as seen in surface view may be compared with the long ovoid form of similar nuclei when seen in cross section.

Vesicle E of figure 23 (rat No. 100, 6 days) presents a stage that is slightly older than the other four vesicles shown in this figure. The floor of this vesicle, the germinal disc, as seen in cross section, presents the form of a triangle with its base resting on the cavity, the blastocoele. When compared with the slightly younger stages this portion of the vesicle presents an increase in the number of constituent cells, arranged in irregular layers to the number of five in its thickest portion. The thickening is no doubt in part due to the slight lateral compression of the vesicle, but this does not wholly account for it. The cells constituting this thickened germinal disc are for the main of irregular polyhedral form with relatively large nuclei rich in chromatin. A distinct covering layer is not evident. On its under surface there is found a single layer of cells of yolk entoderm. The

thin-walled roof of this vesicle, the parietal or transitory ectoderm, deserves no special consideration, except to state that its variation in thickness, as seen in the section figured, is due to the plane of section—cross or oblique—of different portions of the wall, owing to slight folding. This vesicle I believe to be in stage of development and structure very similar to that shown by Sobotta ('03) in his figure 6, mouse vesicle of the first half of the sixth day, and perhaps also figure 31, of the account of Melissinos, mouse vesicle, end of fourth day, also figure 7 of Jenkinson's article who, however, describes and figures a distinct covering or trophoblast layer.

The cell rearrangement and proliferation resulting in the thickening of the floor or the germinal disc as noted in E, of figure 23, marks the beginning of a much more distinct thickening of this portion of the vesicle, partly due to cell proliferation, in part also due to the rearrangement and enlargement of the constituent cells, during which thickening process this portion of the vesicle grows outward as well as into the cavity of the vesicle, initiating the phenomenon known as the 'inversion of the germ layers' or as 'entypy' of the germ layers, to be discussed as to its anlage in the following section.

LATE STAGES OF BLASTODERMIC VESICLE, BEGINNING OF ENTYPY OF GERM LAYERS

The material at hand is listed in table 7.

TABLE 7

RECORD NUMBER	AGE	NUMBER OF VESICLES
46	6 days, 14 hours	10
54	6 days, 16 hours	9
67	6 days, 16 hours	7
24	6 days, 17 hours	3
90	6 days, 17 hours	6
72	7 days	9
80	7 days	9
92	7 days	6 Total 59

The fixation of the blastocysts of the albino rat obtained during the seventh day after insemination was much more

readily accomplished than in those obtained during the preceding day. Of the 59 vesicles of this stage obtained, many show excellent fixation. The thin wall of the vesicle is no longer so prone to fold as in the preceding stage, and does not readily retract from the uterine epithelium or mucosa, no doubt owing to a distinct adhesion of vesicle wall to the maternal tissue. It is difficult, however, so to orient the vesicles as to obtain sections of a desired plane. The general position of a given vesicle is readily determined, since the enlargement of the uterus marking its location is very evident. The vesicles are located in approximately cylindrical cavities, known as decidual crypts, which are directed toward the antimesometrial border.

These decidual crypts communicate with the lumen of the uterus, which lies eccentric and nearer the mesometrial border, by means of funnel-shaped openings. The decidual crypts or cavities are still lined with uterine epithelium, though this is now much flattened in the immediate vicinity of the vesicle and may be found in part separated from the mucosa of this region. The vesicles are now so placed that in all of them, the thicker portion, the floor of the blastodermic vesicles of younger stages or region of the germinal disc, is directed toward the mesometrial border, thus toward the still patent lumen of the uterus, while the roof of the vesicles is directed toward the antimesometrial border, thus toward the bottoms of the decidual crypts. The general direction of the decidual crypts is in the main at right angle to the long axis of the uterine horn, and directed from the mesometrial to the antimesometrial border. They may deviate, however, from the general direction at various angles and in almost any direction. The decidual crypts as seen in cross section do not as a rule present a circular outline, but appear as slightly compressed from side to side, having thus an oval outline as seen in cross section, with the long axis of this oval space as seen in cross section approximately parallel to the long axis of the uterine horn. Since the direction of the decidual crypts can in uncut material be only approximated, the obtaining of sections cut in a desired plane becomes largely a matter of chance. In a large number of my preparations the contained

vesicles are cut in an oblique plane, which may deviate only a little from the longitudinal or may approach a cross axis, while only a relatively small number of vesicles were cut favorably in the longitudinal plane, and the majority of these are in series cut parallel to the plane of the mesometrium. The vesicles on which the special consideration of this stage is based are reproduced as seen in sections, in figure 24.

Vesicle A, of figure 24 (rat No. 46, 6 days, 14 hours), is drawn from two successive sections. The upper portion of the figure



Fig. 24 Sections of blastodermic vesicles or blastocysts of the albino rat showing the early stages of entopy of the germ disc. $\times 200$. A and B, rat No. 46, 6 days, 14 hours; C, rat No. 54, 6 days, 16 hours; *ect.pl.*, ectoplacental cone or Träger; *ect.n.*, ectodermal node; *p.ect.*, parietal or transitory ectoderm; *v.ent.*, visceral layer of entoderm; *p.ent.*, parietal entoderm.

was drawn under camera lucida from one section, then by superimposing certain of the cells so as to give proper orientation, the lower half of the figure was added from the succeeding section. The slightly oblique plane in which this vesicle was cut made this procedure desirable. This relatively small vesicle seems in excellent state of fixation, as is evident from the symmetrical outline shown by the successive sections of the series. When compared with vesicle E of figure 23, though the two are separated in time of development by only a few hours, it is evident

that a distinct advance in development has taken place. The so-called floor of vesicle A, the region of the germinal disc of former stages, directed toward the mesometrial border, is markedly thickened, resulting in an outgrowth toward the mesometrial border and an ingrowth into the cavity of the vesicle. The outgrowth forms the anlage of the 'Träger' (Selenka) or the 'ectoplacental cone' (Duval), and appears to have developed largely as a result of an increase in size of the more superficially placed cells, since cell proliferation is not marked in this region. It is admitted that the critical stages are here lacking in my material. These stages appear to fall to the early hours of the seventh day, the material for which is lacking.

As may be seen from the figure, the cells constituting the anlage of the ectoplacental cone are of relatively large size with large vesicular nuclei, and are continuous at the base with the parietal ectodermal cells which form the roof of the vesicle or its antimesometrial portion. In the cell mass which extends into the cavity of the blastodermic vesicle or blastocyst in which there is recognized the anlage of the 'egg-plug'—'Eizapfen,' or 'egg cylinder'—'Eicylinder' (Sobotta) there is evident a fairly clearly circumscribed compact mass of cells, which stain somewhat more deeply than the surrounding cells and which may be designated as the ectodermal node. It represents the anlage of the true ectoderm of the embryo, as may here be stated in anticipation of further description. In all of the vesicles of this stage of development, even when cut obliquely or in cross section, this small nodule of compactly arranged cells is evident. It is circumscribed both from the cells of the ectoplacental cone as also from the cells lining the blastocele. The metamorphosis leading to the formation of the ectodermal node will receive consideration in a brief general discussion of this stage. The cells covering the egg-plug, and surrounding the ectodermal node, so far as it extends into the blastocele, are arranged in a single layer, forming a dome-shaped membrane, which appears as forced into the cavity of the vesicle consequent on development of the ectodermal node. This layer of cells constitutes the yolk entoderm, the anlage and differentiation of which has been previously

considered. The antimesometrial portion of this vesicle, its roof, consists of a single layer of somewhat flattened cells, the parietal or transitory ectoderm. The parietal ectoderm presents on its inner surface a few—four in the section figured—entodermal cells of irregular outline. These may be designated, after Sobotta, as cells of the parietal entoderm.

Vesicle B, of figure 24, taken from the same rat as was vesicle A (rat No. 46, 6 days, 14 hours) presents a very favorably cut vesicle, which, however, is slightly compressed from side to side, so that its form appears more nearly circular in the sections cut in the plane of the figure, than were they cut at right angles to this plane. This is especially true of the ectoplacental cone, which for the greater part appears in only two sections of 10 μ thickness, while in the plane of the figure it measures nearly 90 μ . Cognizance of this is to be taken in considering the relative size of the ectoplacental cone as shown in this figure. This vesicle is only very slightly older than that shown in A of this figure. Its ectoplacental cone is made up of a core of relatively large cells, bordered by more flattened cells, which in this preparation stain somewhat more deeply than do the more centrally placed cells. These covering cells are continuous with the cells of the parietal ectoderm. The cell mass projecting into the blastocoele is more definitely circumscribed than in the slightly younger stage shown in A of this figure. The ectodermal node appears as an oval mass composed of compactly arranged cells, and is separable on all sides from the surrounding cells. The yolk entoderm, which may now be known as the visceral layer of the entoderm (Sobotta) passes as a single layer of cells of quite regularly cubic or short columnar form, nearly about the ectodermal node to reach the base of the ectoplacental cone, extending over on the parietal ectoderm at one side (see right side of figure). A few of the cells of the parietal entoderm, three in the figure, are evident. The parietal ectoderm forming the roof or antimesometrial portion of this vesicle consists of a single layer of flattened cells, which rest on, and are adherent to the decidual tissue; the uterine epithelium lining the decidual crypt in which the vesicle is lodged having in part disappeared in the immediate region of the vesicle.

Vesicle C of figure 24 (rat No. 54, 6 days, 16 hours) presents a stage which is almost identical in development with that shown in B of this figure, though in shape these two vesicles, as seen in sections, appear quite different. The vesicle shown in C is less compressed than the one shown in B, and probably presents more correctly the form of the blastodermic vesicle or blastocyst of the albino rat at this stage of development. The ectoplacental cone presents a cylindrical outline and contains two cells showing mitotic phases, both included in the section figured. Its cells, more particularly the ones bordering the periphery, present a vacuolated protoplasm, the vacuoles containing lightly colored globules which from reaction to the stain are to be regarded as blood cells or fragments of such, which blood cells are regarded as of maternal origin. In this preparation, the decidual crypt contains a small amount of extravasated maternal blood, found in part surrounding the ectoplacental cone; also in the antimesometrial portion of the crypt in relation with the roof of this vesicle. These findings will receive further consideration in the succeeding pages. The cell mass projecting into the cavity of the vesicle, consisting of the ectodermal node and the layer of visceral entoderm is slightly larger than in the preceding stage but presents no special features deserving discussion. The vesicle in the section sketched presents very few cells of the parietal entoderm. The parietal ectoderm forming the roof of this vesicle consists of a single layer of flattened cells in the protoplasm of certain of which vacuolization is evident. Certain of the cells show inclusions of lightly staining globules of a color similar to those found in the cells of the ectoplacenta, particularly evident in the lower right of the figure in which they are represented as uncolored circumscribed areas. The color reaction of these globules is like that of the maternal blood cells and fragments of blood cells found in the decidual crypt in the immediate vicinity of the vesicle, and they are regarded as blood cells or fragments of such, taken up by the cells of the parietal ectoderm at this stage in the development of the vesicle.

The blastodermic vesicles or blastocysts figured in figure 24, represent an important stage in the development of the albino

rat, as also in a number of other rodents, in that they show the anlage of the phenomenon known as the inversion of the germ layers or entypy of the germ layers. "Inversion of the germ layers—Blätterumkehrung"—in the ova of rodents was probably first recognized by Reichert in the guinea-pig, mouse, and rat, though it was much more fully and correctly described by Bischoff as observed in the guinea-pig and a little later by Hensen, also in the guinea-pig. Further observations on this phenomenon were recorded by Kupffer in his study of the development of the field mouse, *Arvicola arvalis*, and by Fraser on the gray and white rat and the mouse. Selenka gave this question special study, and in a number of monographic communications deals with the phenomenon of Blätterumkehrung as observed in three varieties of the mouse, the white rat, and the guinea-pig. Selenka's observations have formed the basis for future work on this problem. They have been widely accepted and extensively quoted. It was he who introduced the term 'Träger' to denote the cell mass which results from proliferation of the covering cells. His own words concerning this point read as follows:

Während bei dem Kaninchenei, nach erfolgter Sonderung der formativen Furchungszellen in äusseres Ektoderm und inneres Entoderm, die gesammte Lage der äusseren Deckzellen zu einer dünnen resistenten Membran zusammenschrumpft, verdickt sich bei den Nagern mit invertirten Keimblättern der mit den formativen Zellen in Contact befindliche Abschnitt der Deckschicht unter lebhafter Zellvermehrung zu einem sphärischen oder konischen Gebilde, welches ich als 'Träger' bezeichne; * * * * Die Einwucherung dieses Trägers ins Innere der Keimblase hat zur Folge, dass die scheibenförmigen Grundblätter (Ektoderm und Entoderm) sich nicht wie beim Kaninchen zu zwei concentrischer Hohlkugeln erweitern, sondern, ehe sie noch zu dieser Gestalt gelangten, ins Centrum der Keimblase vorgeschoben, vorgestülpt und damit invertirt werden.

In a later publication, this observer also suggested the name 'Entypie des Keimfeldes' as a more comprehensive term than 'Blätterumkehrung' under which may be included types with inversion of the germ field without actual inversion of the germ layers. In later years Duval, Christiani, Robinson, Jenkinson, Sobotta, Kolster, D'Erchia, Spee, Burckhard, Melissinos, Widakowich, Lee and others have studied the earlier developmental

stages of rodents presenting the so-called inversions of the germ layers. O. Hertwig in his chapter "Die Lehre der Keimblätter" gives a brief résumé of our knowledge of the inversion of the germ layers as observed in certain rodents, noting that three main modifications are to be observed. The first and simplest, as found in the field mouse; the second or intermediate as found in the rat and mouse; the third and most complex as observed in the guinea-pig. Hertwig's account is based largely on the observations of Selenka, the accuracy of which is now questioned from many sides.

My own conclusions concerning the early stages of the entypy of the germ layers in the albino rat are made on stages which do not portray the very beginning of this process. The vesicles shown in figure 24, in which this process is well initiated, however, present appearances, on the basis of which certain conclusions may be drawn. It is the contention of Selenka that the Träger or ectoplacental cone is developed as a result of proliferation of covering or Rauber's cells, superimposed on the formative cells of the germ disc. He is followed in this view by Jenkinson, who states that "At a certain stage this proximal trophoblast (the so-called Rauber's cells of the rabbit) certainly becomes very thin, but it never wholly disappears, and soon thickens again to form the Träger, or, to use a modern expression, trophoblastic syncytium, which is destined to play an all-important part in the formation of the placenta." The account of Melissinos is difficult to follow, owing to his application of the term 'Rauber-sche Schicht.' The outer layer of the blastocyst in the region of the germinal disc is said to have a transitory existence and to disappear almost completely in the earlier stages of blastocyst formation. In a later paragraph he states, "dass nur die Rauber-sche Schicht existiert und sogar in den folgenden Stadien mit zahlreicheren Kernteilungsfiguren, und dass sie den Placentar-conus liefert." Attention has previously and on a number of occasions been called to the fact that in the albino rat I have not been able to differentiate a distinct covering layer—Deckschicht or Rauber's Schicht (Selenka); trophoblast layer (Jenkinson)—and have expressed myself as wholly in accord with Sobotta's

observations on the mouse egg as concerns this point. He has critically reviewed Selenka's and Jenkinson's contentions as to the participation of the covering layer in the formation of the Träger or ectoplacental cone, reaching the conclusion that there is no evidence in support of this. In accord with Duval—and in this I concur—he states: “Die mesometrale Spitze des ‘Trägers Selenkás’ ist, wie auch Duval richtig bemerkt, sogar ganz auffällig arm an Mitosen.” The anlage of the ectoplacental cone or Träger, it would appear to me, is primarily the result of enlargement of its constituent cells, this enlargement of cells involving the more peripherally placed cells of the somewhat thickened germinal disc. In none of my preparations showing early stages in the formation of this structure are mitotic figures evident. Grosser in his figures 67 and 113, shows a germinal vesicle of the albino rat of $6\frac{1}{2}$ days in its normal position in the decidual crypt. The vesicle there figured is about identical in time and stage of development to those figured by me in figure 24. In his figures, the Träger (*Tr.*) is represented as consisting of relatively few cells in which no mitoses are evident. In slightly older stages after the means of nutrition of the vesicles is improved through ingestion of maternal blood cells (Sobotta) mitotic figures may be observed in the ectoplacental cone, as shown in C of figure 24. In the rat as in *Mus sylvaticus* and the guinea-pig (Selenka) the ectoplacental cone arises as a solid mass of cells; in *Arvicola arvalis* (Kupffer) it is at first a hollow structure and is in part formed by invagination; in the white mouse (Sobotta) the form of this cell mass may vary greatly and may be solid or penetrated by a mere slit or again by a more extensive cavity.

The earlier stages in the formation of the egg-plug or egg-cylinder I have not been able to follow. In the youngest stage showing this, at my disposal, A of figure 24, it consists of a central node of compactly grouped cells, of polyhedral form, quite definitely demarked from the surrounding cells, and very generally of oval form. This mass of cells I have designated the ectodermal node. In Grosser's figures (67 and 113, e, *Ec*) an identical structure may be observed, designated as ‘Ectoderm der Em-

bryonanlage.' The same may perhaps be observed in figure 26, plate 14, of Selenka's account. In figures 26, 28, 31, and 33 of Christiani's contribution this may be postulated, though his figures are useless for a close comparison. Duval does not figure this stage. Sobotta's ('03) figure 7, and figure 33 of the contribution of Melissinos, appear to give a corresponding stage for the mouse, but in neither of these figures is the 'ectodermal node' so clearly depicted as in Grosser's and my own figures, at least not until a somewhat older stage. Figure 6 of Sobotta ('03) may very probably be regarded as representing an intermediate stage between that shown in E of figure 23 and in A of figure 24. By a proliferation of the cells of the germinal area as shown in the former figure a stage resembling that shown in Sobotta's figure 6, is readily postulated. That the formation of the ectodermal cells is in part due to rearrangement of the cells of the germinal area I believe to be the case, since cell proliferation is not marked in this stage. The enlargement of the more peripheral cells of the germinal area, leading to the anlage of the ectoplacental cone, would of necessity cause the forming ectodermal node to force the yolk entoderm into the cavity of the vesicle, and thus form the anlage of the egg-plug and initiate the phenomenon of entypy of the germ layers. O. Hertwig, in describing the inversion as observed in the mouse and rat, after considering the formation of the Träger through proliferation of the cells of the Deckschicht, following here Selenka's account, states, referring to the Träger, "Durch ihn wird der formative Teil des Ektoblasts nach dem Centrum der Blase vorgetrieben, wobei er sich in eine allseits abgegrenzte Epithelkugel umwandelt." And again, in referring to the development of the guinea-pig, he states: "Wie bei Maus und Ratte zieht sich das formative Ektoderm zu einer Epithelkugel zusammen." Hertwig thus appears to regard the formation of the 'Epithelkugel,' the ectodermal node, as in part at least developed owing to a rearrangement of the cells of the germinal disc. After the formation of the egg-plug or egg-cylinder that portion of the yolk entoderm which covers it is designated by Sobotta as the visceral layer of the entoderm. The scattered entodermal cells, attached

here and there to the inner surface of the parietal ectoderm, in the albino rat at no time forming a continuous layer, he has designated as the parietal entoderm. He is followed in this by Widakowich. This nomenclature has been used by me in the sense employed by Sobotta. The parietal or transitory ectoderm (Kolster's 'feinfassrige Haut') forming the roof or antimesometrial portion of the vesicles, is constituted of a single layer of flattened cells, which in the rat show no regional differentiation. The resorption of maternal blood, incidentally noted with reference to cells of the ectoplacental cone and certain of the cells of the parietal ectoderm in connection with vesicle C of figure 24, to which phenomenon attention has been drawn by Sobotta and Kolster for the mouse, will receive further consideration in the discussion of older stages.

DEVELOPMENT AND DIFFERENTIATION OF THE EGG-CYLINDER

The material at hand is listed in table 8.

TABLE 8

RECORD NUMBER	AGE	NUMBER OF OVA
17	8 days, 17 hours (?)	2 (not all cut)
35	8 days, 18 hours (?)	6
21	7 days, 16 hours	10
66	7 days, 16 hours	7
27	7 days, 17 hours	7
89	7 days, 20 hours	5
81	7 days, 22 hours	7
94	8 days	7
95	8 days	9
96	8 days	5

For the stages showing the development and differentiation of the egg-cylinder in the albino rat I am able to present a series of stages which follow one another in close succession. The figures presented are in themselves so elucidative that an extended description is obviated. The stages under consideration fall within the eighth day after the beginning of insemination, judging from the great majority of the specimens at my disposal, although two rats (Nos. 17 and 35) killed in the latter

half of the ninth day, contained stages which are younger than nearly all of those obtained the latter half of the eighth day. I am unable to state whether this is owing to a retardation in the rate of development of the ova in rats Nos. 17 and 35, or due to an error of record. The record gives date and hour of insemination and of killing, and I have no reason to doubt its accuracy. However, the two rats in question give the only instances of marked deviation from what appears as a normal rate of development as presented by the bulk of my material. Sobotta ('11) has called attention to the difficulty of obtaining successively staged material in the mouse, and cites Kolster as contending: "Man könne auf die Altersbestimmung gar nichts geben." During this stage of development the decidual crypts lodging the ova are deeper than in the preceding stage, their mesometrial portion being narrower, though they are not as yet separated from the uterine lumen. The orientation of the decidual crypts and the contained egg-cylinders is perhaps more readily made than in slightly younger stages, though not definitely enough to insure the cutting of sections in a given plane. Sections of the egg-cylinder cut in the longitudinal plane may be obtained by cutting parallel to the plane of the mesometrium or at right angles to the same. However, it is still largely a matter of chance as to whether the sections obtained pass through the midplane or at an angle thereto.

In figure 25, there are reproduced representative sections of three germinal vesicles taken from the same uterus (rat No. 35, 8 days, 18 hours) which show three closely approximated early stages in the development of the egg-cylinder. None of these three vesicles is cut in exactly the mid-longitudinal plane; especially is this true of the ends of the vesicles. Furthermore, the antimesometrial portion of each, lower part of the figure, composed of the thin-walled parietal ectoderm, shows a certain amount of folding, so that a portion of each wall is cut *en face* instead of *en profile*. The appearances here presented by the antimesometrial portion of these vesicles is not to be confused with a 'giant cell' formation of this portion of the roof of the vesicle, described by Sobotta in his earlier publications, but corrected and retracted

in his later communications. Vesicle A, figure 25, when compared with vesicle C of figure 24, shows only a slight difference in degree of development. Vesicle A is of more elongated and of more distinctly cylindrical form. Its thin-walled portion (an-

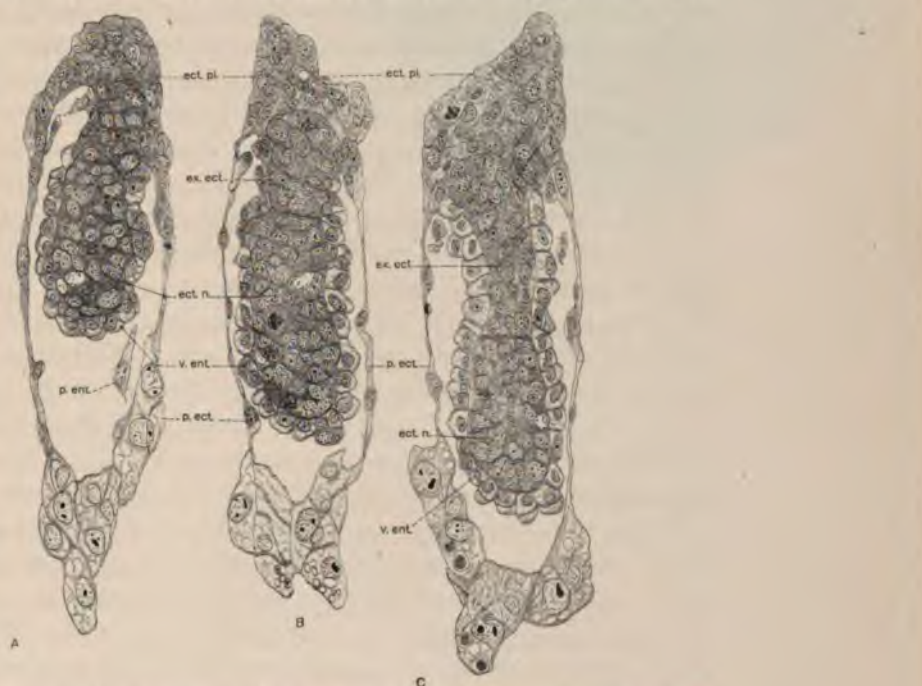


Fig. 25 Longitudinal sections of blastodermic vesicles of the albino rat, showing entypy or inversion of germ layers with early stages in egg-cylinder formation. The ectoplacental cone of each is not cut through its entire length and the lower portion of each vesicle is slightly folded. $\times 200$. A, B, and C, rat No. 35, 8 days, 18 hours, after insemination. To fit properly into the entire series these three vesicles should be from the early hours of the seventh day after insemination. *ect.pl.*, ectoplacental cone or Träger; *ect.n.*, ectodermal node; *ex. ect.*, extraembryonic ectoderm, early stage of its ingrowth shown in vesicle A; *p. ect.*, parietal or transitory ectoderm; *v.ent.*, visceral layer of entoderm; *p.ent.*, cells of parietal entoderm.

timesometrial portion) is longer, its cavity more extensive; this is owing to a further flattening of the cells of the parietal or transitory ectoderm. In vesicle A in the section preceding the one figured, the ectoplacental cone is thicker by about two

rows of cells than in the one figured; the section figured not passing through the center of this structure. In vesicle A, the ectodermal node, which is distinctly demarked, no longer rests against the base of the ectoplacental cone, as in C of figure 24, but has been forced farther into the cavity of the vesicle by reason of proliferation of the cells at the base of the ectoplacental cone, resulting in the formation of a nearly cylindrically formed column of compactly arranged, polyhedral-shaped cells interposed between the ectodermal node and the base of the ectoplacental cone, but merging into the latter without sharp demarcation. To this mass of cells the name of extraembryonic ectoderm has been given by Widakowich. However, under this term this author includes also the cells of the ectoplacental cone. The ectodermal node is of larger size than in the slightly younger stage, C of figure 24, the result of cell proliferation. In the section sketched, three mitotic figures are evident in this structure. Its cells are of polyhedral shape, and show no definite arrangement. The ectodermal node and the extraembryonic ectoderm, to the base of the ectoplacental cone, together form a cylindric structure enclosed within a layer of visceral entoderm, which in the section figured is in part cut tangentially, and thus simulates an epithelium consisting of two layers of cells, but consisting in reality of a single layer of cells. Ectodermal node, extraembryonic ectoderm, and the layer of visceral entoderm together form a structure of cylindric shape which extends into the cavity of the vesicle for a distance about one-half its extent, forming the anlage of the egg-cylinder (Sobotta). Very few parietal entodermal cells are to be found on the inner surface of the parietal ectoderm. Vesicles B and C of figure 25 differ from that discussed under A, only to the extent to which the ectodermal node has been forced into the cavity of the vesicle owing to further growth of the extraembryonic ectoderm, to the extent that in C, the elongated egg-cylinder approaches the antimesometrial end of the cavity of the respective vesicle. Ectodermal node and extraembryonic ectoderm are at this stage distinctly demarked, though in close apposition. An indenture from the surface at the region of the union of these structures

with a consequent infolding of the layer of visceral entoderm is not as a rule evident, if so, only very slightly, as to the left in B; such infolding of the visceral entoderm is not regarded as having special significance. These structures, ectodermal node and extra-embryonic ectoderm, are appropriately referred to as ectodermal cylinder by Widakowich, and with the visceral entoderm, as constituting the egg-cylinder of Sobotta.

Under A of figure 26 (rat No. 17, 8 days, 17 hours), there is shown a representative section of a vesicle which is only very slightly older than that shown under C, figure 25. This vesicle was exposed, by teasing away, after fixation, the decidual tissue forming one side of the decidual crypt; this being done before embedding, so as to admit of orientation of its long axis. This accounts for the collapsed state of the thin wall of the vesicle and its slight folding, also for the fact that the ectoplacental cone is reflected upon itself. The egg-cylinder is cut in a very favorable longitudinal plane. In its antimesometrial portion, lower part of the figure, the cells of the ectodermal node now show definite arrangement in practically a single layer, with alternating nuclei. The beginning of a central cavity is evident with reference to which the cells are arranged. This cavity is the anlage of the 'Markamnionhöhle' of Selenka, more appropriately known as the antimesometrial portion of the proamniotic cavity. The cells forming the wall of the ectodermal vesicle (Ektodermblase, Selenka), derived from the ectodermal node, may now be known as the primary embryonic ectoderm (Widakowich). The extraembryonic ectoderm in the mesometrial portion of the egg cylinder has differentiated to form a relatively long irregularly cylindric structure, continuous with the base of the ectoplacental cone, composed of irregular polyhedral cells, compactly arranged and showing as yet no definite orientation. In these cells active proliferation is evidenced by numerous mitoses. The egg-cylinder is covered by a single layer of cells of the visceral entoderm. Over the antimesometrial end of the egg-cylinder, the entodermal cells now present a cubic or thick pavement form, while along the sides of the egg-cylinder they are of columnar form, especially long in the region where the primary



Fig. 26 Longitudinal sections of egg-cylinders of the albino rat, showing the anlage of the antimesometrial and mesometrial portions of the proamniotic cavity. $\times 200$. A, rat No. 17, 8 days, 17 hours; B and C, rat No. 81, 7 days, 22 hours, after insemination. A, shows the very beginning of the development of the antimesometrial portion of the proamniotic cavity developing within the ectodermal node; C shows the beginning of the proamniotic cavity developing in the extraembryonic ectoderm; *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *ex.ect.*, extraembryonic ectoderm; *v.ent.*, visceral entoderm in B and C, the cells of this layer showing the anlage of the three zones showing absorption of maternal hemoglobin; *a.met.pr.*, antimesometrial portion of proamniotic cavity, developing in the ectodermal node; *pr.emb.ect.*, primary embryonic ectoderm; *ect.yes.*, ectodermal vesicle; *met.pr.*, mesometrial portion of the proamniotic cavity, developing in the extramebryonic ectoderm.

embryonic ectoderm and the extraembryonic ectoderm meet. The special cytomorphosis undergone by the columnar cells of the sides of the egg-cylinder, in contradistinction to those of the antimesometrial end, will be considered in later pages. The visceral layer of the entoderm extends to the base of the ectoplacental cone, in part passing over onto the layer of parietal ectoderm. In the section figured, cells of the parietal layer of the entoderm are not evident. The ectoplacental cone has grown in length in the direction of the lumen of the uterus or the mesometrial border. In the great majority of my preparations this structure is slightly compressed from side to side, so as to be broader in a plane parallel to the long axis of the uterus. In vesicle A, it is cut at right angles to the long axis of the uterus, thus appears as much narrower than in the other two vesicles of figure 26, which were cut in a plane parallel to the plane of the mesometrium. The increase in size of the ectoplacental cone is the result of active cell proliferation. Mitotic figures to the number of one, two or three, may now be observed in nearly every section of this structure. The parietal or transitory ectoderm, continuous with the base of the ectoplacental cone, has been reduced by this stage to a thin, practically homogeneous membrane, presenting scattered, flattened nucleated cells on its inner surface. This thin membrane is now quite firmly adherent to the wall of the decidual crypt, throughout nearly its whole extent.

Under B of figure 26 (rat No. 81, 7 days, 22 hours) there is shown a representative section of a vesicle which is slightly more advanced in development than that shown in A of this figure. The antimesometrial portion of the proamniotic cavity, the anlage of which was shown in the preceding stage, is well established. Its wall, consisting of primary embryonic ectoderm is composed of a single layer of cells with nuclei in essentially the same plane. The primary embryonic ectoderm forms a closed vesicle (Ectodermblase, Selenka) distinctly demarked from the extraembryonic ectoderm. In this as in the preceding stage the extraembryonic ectoderm forms a long cylindrical structure continuous at its mesometrial end with the base of the

ectoplacental cone. The cells are of irregular polyhedral form, compactly grouped, showing as yet no definite arrangement. Cell proliferation as evidenced by mitoses is active, amply accounting for the increase in length of this structure. The visceral entoderm encloses the long egg-cylinder as a single layer of cells and is continuous at its base with the parietal entoderm, well shown at the left of the figure. The ectoplacental cone of this vesicle is very favorably cut in a plane parallel to the long axis of the uterus. This vesicle was unusually well fixed and may be regarded as showing normal relations of the thin membranous wall, derived from the parietal ectoderm, and of the egg-cylinder, which reaches quite to the antimesometrial end of the vesicle.

Vesicle C of figure 26, obtained from the same uterus as was vesicle B (rat No. 81, 7 days, 22 hours), differs from that shown under B, in that it presents the anlage of a mesometrial portion of the proamniotic cavity. In the extraembryonic ectoderm, near its junction with the base of the ectoplacental cone, two irregular spaces may be observed. These are distinctly evident, passing through the entire section, only in the section figured. The antimesometrial portion of the egg-cylinder is not cut quite through its center, so that the primary embryonic ectoderm of the ectodermal vesicle appears as a stratified epithelium, and the antimesometrial portion of the proamniotic cavity appears as relatively small, this owing to a slight curvature shown by this egg-cylinder. The other features presented by this vesicle are sufficiently well portrayed in the figure to obviate the necessity of further description.

In figure 27, there are shown three further stages of egg-cylinder differentiation, showing progressively older stages than shown in the preceding figure. Under A of this figure, there is reproduced a representative section of a vesicle taken from the same uterus as were vesicles B and C of figure 26 (rat No. 81, 7 days, 22 hours). The figure is not of a single section, but is combined from two sections, superimposed so as to give correct dimensions and relations. The egg-cylinder of A of this figure differs from that shown in C of figure 26, in that the mesometrial



Fig. 27 Longitudinal sections of egg-cylinders of the albino rat showing fusion of the antimesometrial and the mesometrial portions of the proamniotic cavities. $\times 200$. A, rat No. 81, 7 days, 22 hours; B, rat No. 96, 8 days; C, rat No. 94, 8 days, after insemination; *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *ex.ect.*, extraembryonic ectoderm; *ect.ves.*, ectodermal vesicle, with wall composed of primary embryonic ectoderm, at + junction with the extraembryonic ectoderm; *a.met.pr.*, antimesometrial portion of proamniotic cavity; *met.pr.*, mesometrial portion of proamniotic cavity; *pr.c.*, proamniotic cavity; *v.ent.*, visceral entoderm; *pr.emb.ent.*, primary embryonic entoderm.

portion of the proamniotic cavity, developing in the extraembryonic ectoderm, is of greater dimension. Two relatively large spaces, bordered by a single layer of cells of the extraembryonic ectoderm, are to be observed. At the junction of the extraembryonic ectoderm and the ectodermal vesicle of primary embryonic ectoderm a further space of triangular outline may be seen. The primary embryonic ectoderm is arranged in the form of an oval-shaped vesicle, forming the antimesometrial end of the egg-cylinder. Its wall is relatively thin at the region of its apposition to the extraembryonic ectoderm, just below the triangular space above mentioned. This ectodermal vesicle is peculiar in that its cavity contains the remains of four cells. A study of the series of sections shows that these cells do not represent the crest of a fold of the wall of this vesicle, since they are not nearly so distinct in preceding and succeeding sections. It may only be conjectured that during the rearrangement of the cells of the ectodermal node, resulting in the formation of the ectodermal vesicle, certain of the cells became separated from the wall and remained free in the cavity. The primary embryonic ectoderm, forming the wall of the ectodermal vesicle is readily differentiated from the extraembryonic ectoderm, both by the fairly sharp definition of the ectodermal vesicle and by reason of the fact that its cells stain somewhat more deeply than do the cells of the extraembryonic ectoderm, as also the cells of the visceral entoderm. In the egg-cylinder shown under B of figure 27 (rat No. 96, 8 days) the antimesometrial portion of the proamniotic cavity, developing in the ectodermal node, and the mesometrial portion of the proamniotic cavity, developing as several discrete spaces in the extraembryonic ectoderm, have in part joined to form a single proamniotic cavity. The mesometrial portion of this cavity is still bridged by a septum of extraembryonic ectodermal cells, closing off a relatively large space found in its mesometrial portion. With the junction of the antimesometrial and the mesometrial portions of the proamniotic cavity, the primary embryonic ectoderm and the extraembryonic ectoderm become a continuous layer, the line of union of the two portions, however, remains evident and is readily recognized in all the egg-cylinders

of this and older stages, a question which will receive further consideration in following pages.

In C of figure 27 (rat No. 94, 8 days) the proamniotic cavity forms a continuous, single space. The figure presented is drawn from two sections; its greater portion, to the base of the ectoplacental cone from one section, the ectoplacental cone from another section. The junction of the membranous wall of the vesicle to the base of the ectoplacental cone, in the two sections used for the figure, was superimposed under camera lucida in joining the portions drawn from the two sections. It is believed that the drawing as presented gives correctly dimensions and relations of the different parts of this vesicle. The wall of the antimesometrial portion of the single proamniotic cavity is formed by the primary embryonic ectoderm, the cells of which are for the main of irregular columnar shape, with alternately placed nuclei. These cells are in active proliferation, as is evidenced by numerous mitoses. The wall of the mesometrial end of the proamniotic cavity is formed of a single layer of cells of the extraembryonic ectoderm; these cells are of quite regular shape with nuclei placed in about the same plane. They stain less deeply than do the cells of the primary embryonic ectoderm. In this egg-cylinder (C, fig. 27) the proamniotic cavity does not extend so near the base of the ectoplacental cone as in a number of other preparations in my possession, showing about the same stage of development; in certain of these, the proamniotic cavity extends to near the mesometrial end of the egg-cylinder.

A more definite characterization of the different parts of the egg vesicle of the albino rat at the stage of development shown in C, figure 27, end of the 8th day, seems desirable, and in doing so I shall use the terminology used by Sobotta and Widakowich. The vesicle under consideration has reached a length of 0.65 mm., and a width of 0.12 mm. Somewhat more than one-fourth of its length consists of ectoplacental cone or Träger. The cavity enclosed is derived from the cavity of the blastodermic vesicle with germ disc, the blastocele, and is termed by Sobotta and Widakowich the 'Dottersackhöhle' or yolk-sac cavity. This cavity is bounded by a thin structureless mem-

brane derived from the parietal or transitory ectoderm and the scattered cells forming the parietal layer of entoderm. This membrane is continuous with the base of the ectoplacental cone and presents scattered flattened cells on its inner surface. I have designated this thin membrane with cells on the inner surface as the parietal or transitory ectoderm (Kolster's *feinfaserige Haut*). The egg-cylinder which extends to the antimesometrial end of the yolk-sac cavity, encloses the proamniotic cavity, the antimesometrial portion of which is walled by primary embryonic ectoderm, its mesometrial portion by extraembryonic ectoderm, the two forming a continuous layer, with line of union of the two types of ectoderm evident. The uncleaved extraembryonic ectoderm is continuous with the base of the ectoplacental cone. The egg-cylinder is surrounded by a single layer of cells of the visceral entoderm, differentiated so as to consist of a portion which surrounds the antimesometrial end of the egg-cylinder in relation with the primary embryonic ectoderm; the cells of this portion being of a rather thick pavement type, constituting the primary embryonic entoderm, and further a portion which covers the sides of the egg-cylinder, with cells of a columnar type, showing special cytomorphosis. The egg-vesicles and egg-cylinders of the stage of development under consideration and for somewhat older stages show no bilateral symmetry so far as can be discerned by study under the microscope. In longitudinal sections of egg-cylinders, cut respectively in two different planes, at right angles to each other, no difference in form, relation and structure of different parts can be observed. Selenka, Kupffer, Duval, and Sobotta have previously called attention to this fact and shown that longitudinal sections of egg-cylinders may be obtained no matter whether the sections are cut parallel to the plane of the mesometrium, thus parallel to the long axis of the uterus, or at right angles to this plane. The want of bilateral symmetry is also evident in cross sections of the egg-cylinder, as may be seen from the series of sections presented in figure 28 (rat No. 27, 7 days, 17 hours). The cross-cut egg-cylinder, from several sections of which these figures were drawn, represents a stage of develop-

ment very similar to that of the egg-cylinders shown in longitudinal section in figure 26.

Widakowich, after discussing very briefly the mode of development of the egg-cylinder, discusses and figures an egg-cylinder of the albino rat, obtained $6\frac{3}{4}$ days after the last coitus. His figure 3 corresponds in stage of development very closely to that shown by me in A of figure 27. In his figures, there is presented an egg-cylinder showing the anlage of the mesometrial



Fig. 28 A series of cross sections at different levels of an egg-cylinder of the albino rat after the anlage of the antimesometrial portion of the proamniotic cavity. $\times 200$. Rat No. 27, 7 days, 17 hours, after insemination. The sections selected for the several levels drawn, A to D, are as follows: A, middle of ectoplacental cone; B and C, through extraembryonic ectodermal portion of egg-cylinder, just below junction with ectoplacental cone (B), and just above ectodermal vesicle (C); D, through middle of ectodermal vesicle. Compare with B, figure 26, a longitudinal section of an egg-cylinder of the same stage of development; *p.ect.*, parietal or transitory ectoderm; *ex.ect.*, extraembryonic ectoderm; *pr.emb.ect.*, primary embryonic ectoderm of the ectodermal vesicle; *v.ent.*, visceral entoderm; *pr.emb.ent.*, primary embryonic entoderm; *a.met.pr.*, antimesometrial portion of proamniotic cavity.

portion of the proamniotic cavity. Emphasis is given to the fact that in the antimesometrial portion of the egg-cylinder, there may be recognized the primary embryonic ectoderm. His own words with reference to this point read as follows:

Der Schnitt zeigt nun sehr deutlich, dass sich die Zellen, die die antimesometrale Höhle so begrenzen, dass die alte Kugel-oder Eiform dieses Teiles noch zu erkennen ist—das primäre embryonale Ectoderm—intensiver färben wie die Zellen des mesometralen Abschnittes oder die des Ectoplacentalconus—das extraembryonale Ectoderm. Die Kerne zeigen keinerlei Unterschied in der Färbung, wohl aber das Plasma, dass im antimesometralen Teile von dichterem Structur zu sein scheint.

This description corresponds very closely to that given by me for a similar stage. The differentiation of these two kinds of ectoderm was also recognized by Robinson, who states:

The epiblastic cylinder is closed at its distal end, the trophoblastic at its proximal, and the open ends of the two cylinders are in close apposition, but not indistinguishably fused, for the character of each portion of the ectoderm, after treatment with carmine, is still quite distinctive; the protoplasm of the trophoblast being tinged much more faintly than that of the epiblast.

Selenka, on the other hand, who has recognized in his 'Ektodermblase' with 'Markamnionhöhle' a distinctive structure, believes this to blend completely with the Träger. Since his account with reference to this point has influenced later workers, I may be permitted to quote him in the original. Referring to the 'Ektodermblase' with 'Markamnionhöhle,' he states:

Dieser Ektodermkeim, welcher von dem vorrückenden Trägerzapfen anfänglich sehr wohl abgegrenzt ist, indem beiderlei Gebilde sich in Folge der convexen Krümmung ihrer einander zugekehrten Flächen sozusagen nur in einem Punkte berühren, fließt endlich mit dem Träger vollständig zusammen, und zwar bei der Waldmaus bevor, bei der Ratte und Hausmaus aber nachdem die Markamnionhöhle entstanden war.

That the proamniotic cavity of the egg-cylinder of the albino rat has its anlage in two distinct cavities, the one developing in the ectodermal node in the antimesometrial portion of the egg-cylinder, which is the first to develop; the other in the mesometrial portion in the extraembryonic ectoderm, was recognized by Selenka (fig. 30, plate 14, E, Markamnionhöhle, E', falsche Amnionhöhle), Duval (fig. 100,) Robinson, and Widakowich (fig. 3). Corresponding stages of egg-cylinder development as presented by me in figures 26 and 27, for the albino rat, are shown by Sobotta ('02), for the mouse in his figures 12 to 14 and text figures a to f. On comparison of my figures with Sobotta's, it becomes evident that the egg-cylinder of the rat is much longer and more slender than that of the mouse. According to the account of Sobotta, the egg-cylinder of the mouse, soon after its anlage, shows by reason of a distinct transverse furrow a division into two parts, an antimesometrial portion of globular form, surrounded by a visceral layer of entoderm, corresponding to what I have designated as the ectodermal node; and a mesometrial portion which early shows the anlage of a proamniotic

cavity. A lumen is obtained in the antimesometrial portion later than in the mesometrial portion. As development proceeds, this sharp demarkation of antimesometrial and mesometrial portion is gradually lost. This, as stated in his own words, reads:

Sehen wir von dem die (der Keimhöhle zugekehrte) Oberfläche des Cylinders überziehenden Dotterentoderm zunächst ab, so sieht man, dass die Furche, welche die oben erwähnten mesometralen und antimesometralen Abschnitte in Stadium der Fig. 11 u. 12 trennte, jetzt wieder wenig deutlich ist. Es bahnt sich eine Verschmelzung beider Abschnitte wiederum an, was man am leichtesten daraus ersieht, dass bald (Fig. 14) beide Abschnitte ein gemeinsames Lumen erhalten.

With the formation of a continuous proamniotic cavity, this is bordered by a single layer of 'ectodermal cells,' with alternately placed nuclei. The cells are described as being the same throughout; neither in text nor figure does Sobotta differentiate between ectodermal cells derived from the antimesometrial portion of the egg-cylinder and those derived from the mesometrial portion. Melissinos also recognizes antimesometrial and mesometrial portions in the development of the egg-cylinder of the mouse, in his figure 34. According to this observer, the antimesometrial portion of the proamniotic cavity is the first to appear; later it appears in the mesometrial portion, the two cavities joining as development proceeds. The parts of the ectoderm derived from these two portions may be recognized, however, after a single proamniotic cavity has developed. This Melissinos states in the following words: "Trotz aller Vereinigung der beiden Höhlungen bleibt die Unterscheidung des normals abgesonderten antimesometralen Abschnittes von dem mesometralen immer leicht zu machen, sei es durch eine klare Grenzlinie oder durch eine an der Peripherie des visceralen Dotterblattes befindliche Furche." The account of Melissinos is more in agreement with the presentations as observed in the albino rat than is that of Sobotta.

Selenka, Sobotta, and Melissinos recognize three different regions of constriction to which significance is given, in the egg-cylinder of the mouse. As stated by Sobotta, the first con-

striction is in the region of the original furrow which demarks the antimesometrial and the mesometrial portions of the egg-cylinder, the region of the primary amniotic fold; the second where the mesometrial cavity ends; and the third where the original blastodermic cavity reaches its mesometrial end. The three folds recognized by Melissinos, are characterized by the specificity of the ectoderm. Since his statement concerning this point is somewhat involved, I find it necessary to use his own words; they read as follows, referring to these folds he states:

Der eine derselben *a* liegt antimesometrial und ist der bekannte erste kugelförmige Buckel (Ektoderm) mit den länglichen, cylinder-pyramidalen oder polygonal-pyramidalen Zellen; der zweite *b* liegt in der Mitte und besteht aus kubisch-polygonalen Zellen, und der dritte Buckel *c*, aus polygonalen Zellen bestehend, liegt mesometrial und ist von dem mittleren durch Einschnürung, von der Basis des Ectoplacentarconus aber durch die bekannte Urfurche des Eicylinders getrennt, in der sich das viscerele Dotterblatt zum parietalen Dotterblatt umbiegt.

So far as I am able to determine, the account of Melissinos agrees with that given by Sobotta, as concerns the folds of the egg-cylinder of the mouse. Selenka's account need not receive special consideration.

In well-fixed egg-cylinders of the albino rat no such folds are recognized. At the line of junction of the primary embryonic ectoderm and the extraembryonic ectoderm, a slight infolding of the layers, variable in degree, is recognized. Other foldings of the wall of the egg-cylinder I have regarded as accidental and not of special significance. Therefore, I am wholly in accord with Widakowich, who has also discussed this question with reference to the albino rat and has described the low fold in the region of the junction of the primary embryonic ectoderm and extraembryonic ectoderm. Referring to that fold, he states: "Dass war die einzige konstante, bald stärker, bald schwächer ausgeprägte Einschnürung der Proamnionhöhle."

Sobotta deserves credit for having described fully the differentiation and cytomorphosis of the cells of the visceral entoderm of the egg-cylinder, and since his observations on this point apply in the main to the albino rat, they may at this time be given

consideration. During the early stages of egg-cylinder differentiation and anlage of the proamniotic cavity, the layer of visceral entoderm differentiates into a portion which is in relation with the primary embryonic ectoderm of the antimesometrial portion of the egg-cylinder, in which region the cells of the entoderm are first of short cubic shape, later of the pavement type; this portion may be regarded as forming the primary embryonic entoderm, since it forms the greater part of the entoderm of the embryo. The greater part of the visceral entoderm, that which surrounds the sides of the mesometrial portions of the egg-cylinder, consisting of extraembryonic ectoderm, differentiates into cells of the columnar type. In this latter portion, with the formation of a continuous proamniotic cavity, the entodermal cells undergo characteristic cytomorphosis. In them, as stated by Sobotta, there may be recognized three main zones: (1) a basal zone with denser protoplasm containing the nucleus; (2) a middle zone with markedly vacuolated protoplasm; (3) an outer zone in which hemoglobin granules are recognized, the latter zone staining deeply in eosin. These three zones in the cells of the visceral entoderm in the region of the extraembryonic ectoderm of the egg-cylinder may be recognized in figures 26 and 27, not so clearly as in Sobotta's colored figures, particularly his figure 17 ('03) and figure 8 ('11). However, I am able to follow closely his description in my own preparations of a somewhat older stage than thus far figured. It is Sobotta's contention that in the extravasated blood surrounding the egg vesicle, in close apposition to its thin outer wall, there may be observed many red blood cells which, though presenting normal form, show a distinctly granular content. These granules stain deeply in eosin and are in shape, size, and reaction to stain very similar to granules found in the peripheral part of the cells of the visceral entoderm. On the outer surface of the thin wall of the vesicle; on its inner surface; in the cells lining this; in the yolk sac cavity; and on the outer surface of the cells of the visceral entoderm, similar granules are found. These appearances are interpreted as showing an absorption of maternal hemoglobin by the entodermal cells of the mesometrial portion of the egg-cylinder.

Sobotta's statement concerning this point, which, owing to its importance, I quote in full, reads as follows:

Man wird diese mikroskopisch erkennbaren Verhältnisse nicht anders deuten können als in folgender Weise: Die Hämoglobinschollen, die durch die äussere Wand des Dottersackes in die Dottersackhöhle gelangt sind, werden von der Oberfläche des zylindrischen, die ganze Seitenfläche des Eizylinders überziehenden visceralen Dottersack-epithels aus resorbiert und zwar geschieht das in der Weise, dass die Hämoglobinschollen zunächst als solche in der Zelle selbst eintreten, dann aber im vacuolisierten Teil der Zelle gleichsam verdaut werden, wobei die einzelnen kleinen Schollen vorher zu grösseren Tropfen zusammenfliessen scheinen.

My own observations on the albino rat as concerns this phenomenon, more particularly as concerns the structure of the cells of the visceral entoderm in the region of the extraembryonic ectoderm, corroborate Sobotta in many particulars. This question will be again and more fully considered in a contemplated later publication dealing with the implantation and decidua formation in the albino rat. It could not be considered now without a discussion of the changes involved in the development of the decidua, a question which I am not prepared to consider fully now. It may be stated, however, that judging from my own preparations and the figures of Grosser, the extravasation of blood into the egg chamber is not nearly so extensive in the albino rat as is shown in the figures of Sobotta for the mouse.

The thin membrane which surrounds the yolk-sac cavity, which I have designated as the parietal or transitory ectoderm, is derived in development from the parietal or transitory ectoderm, and the relatively few parietal entodermal cells, as described and figured for younger stages. At the stage of egg-cylinder development under consideration—with continuous pro-amniotic cavity—this structure appears as a thin, practically homogeneous membrane with scattered, flattened nucleated cells on its inner surface. Sobotta regards these cells as derived from the parietal entoderm, the cells of the parietal ectoderm having disappeared. As concerns this, I am unable to speak with certainty, since the Congo red solution used as a double stain is not particularly favorable in differentially coloring these

cells. However, I am disposed to regard these flattened cells as derived from the parietal ectoderm. The parietal entodermal cells are never numerous in the rat, and mitotic figures are seldom observed in them. With the extension of the vesicle with the enlargement of the blastocele, the cells of the parietal or transitory ectoderm become attenuated until they appear for the greater part as a thin cuticular membrane, and I am disposed to regard the flattened nucleated masses of protoplasm lining the inner surface of this membrane as derived from the cells of the parietal ectoderm.

Much attention has been given to certain large cells which are found in close relation with the outer surface of this thin membrane. These cells, generally referred to as giant cells (*Riesenzellen*) were, by Duval, Sobotta (earlier publications) and Grosser thought to be of embryonic origin and derived from the cells of the parietal ectoderm. Selenka, Disse, Kolster, Melissinos, Pujiula, Widakowich, and later Sobotta ('11) regard them as derived from the maternal tissue and as representing differentiated decidual cells. It is not my purpose to consider more fully these cells in the present communication, since they are by me not regarded as of embryonic origin. My own observations as concerns them agree in the main with those of Widakowich, who, in the albino rat has followed their origin from decidual cells. Since not of embryonic origin, they have been disregarded in making the figures.

I have previously, in connection with a discussion of the structure of vesicle C, figure 24, alluded to the fact that the cells of the ectoplacental cone as also the cells of the parietal or transitory ectoderm have a phagocytic action for maternal blood cells. This Sobotta has also observed for the mouse, in which he is confirmed by Kolster who has further shown that the cells of the ectoplacental cone also take up fat particles. With the ingestion of maternal blood cells by the cells of the ectoplacental cone, more particularly, with the absorption of hemoglobin by the entodermal cells of the mesometrial portion of the egg-cylinder, a period of rapid growth of the egg vesicle is initiated. To this Sobotta has called attention for the mouse; the same

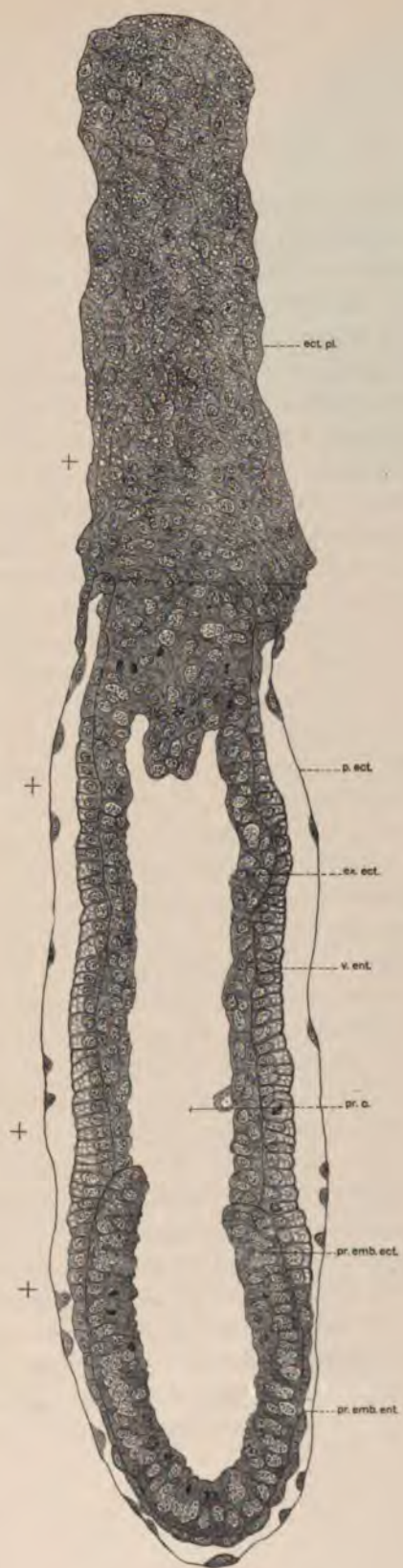
is evident in the albino rat. Indeed, Sobotta presents the far-reaching conclusion that the explanation of the phenomenon of germ layer inversion or entypy of the germ layers is to be found in the dearth of food supply of the ovum in the stages preceding the formation of more definite relations between the ova or germ vesicles with the decidua. It is thought by this observer that the inversion of the germ disc has for its purpose the increase of the absorptive surface of the visceral or yolk sac entodermal epithelium, which as a differentiated layer comes to surround nearly the whole of the egg-cylinder on completion of the inversion, and is thus increased in extent and brought in relatively close relation with the maternal blood lacunae surrounding the egg vesicle.

LATE STAGES IN EGG-CYLINDER DIFFERENTIATION AND THE ANLAGE OF THE MESODERM

In the rat series there are found 24 egg cylinders showing the stages of development considered in this section; certain of them are cut longitudinally and others cross-wise.

For the special consideration of egg-cylinder formation just prior to the anlage of the mesoderm, I present two egg-cylinders obtained during the latter half of the ninth day after insemination; one of these was cut longitudinally, the other in favorable cross-section. The egg-cylinder shown in figure 29, rat No. 40, 8 days, 17 hours after insemination, seems unusually well fixed, as evidenced by its symmetrical outline, and is cut in a very favorable plane. The sections are from a series cut at right angles to the long axis of the uterine horn. The decidual crypts lodging the egg-cylinders of this stage are by this time nearly completely separated from the lumen of the uterus, and are surrounded by a well-developed decidua. Extravasated maternal blood nearly surrounds such egg-cylinders.

Fig. 29 Longitudinal, sagittal section of egg-cylinder of the albino rat showing the final mesoderm-free stage. $\times 200$. Rat No. 40, 8 days, 17 hours, after insemination; *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *pr.emb.ect.*, primary embryonic ectoderm; *ex.ect.*, extraembryonic ectoderm; *pr.c.*, proamniotic cavity; *v.ent.*, visceral entoderm, absorptive for maternal hemoglobin, cells showing the three zones described by Sobotta; *pr.emb.ent.*, primary embryonic entoderm.



The egg-cylinder shown in figure 29 presents a total length of 1.15 mm., a width of approximately 0.18 mm. The ectoplacental cone presents a length of 0.4 mm. and of the proamniotic cavity, 0.5 mm., of which 0.2 mm. falls to the antimesometrial portion lined by primary embryonic ectoderm. This egg-cylinder differs only in shape and size from that shown in C of figure 27, obtained 8 days after insemination. The primary embryonic and extraembryonic ectoderm lining or enclosing the proamniotic cavity are readily differentiated. The primary embryonic ectoderm, derived from the ectodermal node, constitutes a pseudostratified epithelium, composed of relatively long columnar cells, with nuclei radially placed with reference to the lumen of the proamniotic cavity, and shows active cell division, no less than 12 mitotic figures occurring in the section figured. The protoplasm of its cells stains distinctly deeper than does that of the cells of the extraembryonic ectoderm. The cells of the latter are of cubic, short columnar, or polyhedral shape, arranged in a single or double layer, with no definite arrangement of the long axes of its nuclei. It is, therefore, possible readily to distinguish—by reason of shape and size of cells, relative position of nuclei, reaction to stain of protoplasm—between the cells of the primary embryonic and extraembryonic ectoderm, and to determine the sharp line of junction at which the two types of cells form a continuous layer, a fact which will receive further consideration in dealing with the anlage of the mesoderm as observed in slightly more advanced stages. At the mesometrial end of the proamniotic cavity, the cells of the extraembryonic ectoderm become continuous with the cells at the base of the ectoplacental cone; in the region of this junction, active mitosis are often to be observed. In this egg-cylinder the visceral entoderm may readily be differentiated into two portions. The portion which surrounds the primary embryonic ectoderm to nearly the region of its junction with the extraembryonic ectoderm, consists of a single layer of broad, flattened cells which assume a cubic or short columnar shape as the mesometrial border of the primary embryonic ectoderm is approached. This portion of the visceral entoderm we have designated as

the primary embryonic entoderm. The portion of the visceral entoderm surrounding the sides of the egg-cylinder in the region of the extraembryonic ectoderm, to near the base of the ectoplacental cone, consists of a single layer of columnar cells, regularly arranged and presenting the three zones described by Sobotta. In this stage of egg-cylinder development of the albino rat, the absorption of hemoglobin granules derived from maternal blood cells, first shown for the mouse by Sobotta and Kolster, may be readily made out. In preparations stained in hematoxylin and Congo red, in and on the outer zone of the visceral entodermal cells there may be observed granules staining deeply in the Congo red, presenting the color reaction of hemoglobin. In the middle zone of these cells the protoplasm is distinctly vacuolated, while the inner zone, containing the nuclei, presents a denser protoplasm. The transitory or parietal ectoderm consists of a homogeneous membrane, closely adherent to the maternal decidua, especially along the sides of the egg-cylinder. This layer presents scattered nucleated protoplasmic masses of spindle or dome shape on its inner surface, the relations and distribution of which may be clearly seen in the figure. Attention needs yet be drawn to the ectoplacental cone of the egg-cylinder. Its relation to the maternal decidua is very intimate, so that in places, owing to blood extravasations, it is difficult to differentiate between embryonic and maternal tissue. Many of the cells of the ectoplacental cone present a vacuolated protoplasm, the vacuoles enclosing maternal blood cells. Therefore, they are distinctly phagocytic. Sobotta has also observed and described this for the mouse. Referring to a slightly older stage after the anlage of the mesoderm, his own words read as follows:

Weiterhin sehen wir im Stadium der Fig. 5 auch eine starke Verlängerung und Vergrößerung des Ectoplacentarconus, an dem im mesometralen Teile jetzt Vacuolen auftreten, die in späteren Stadien regelmässig gefunden werden und zwar erfüllt mit mütterlichen Blutextravasaten. Die Ernährung des Embryo mit mütterlichem Hämoglobin * * * * ist jetzt im vollen Gang.

Absorption of maternal hemoglobin by the cells of the ectoplacental cone appears to be established at a relatively earlier period in the rat than in the mouse.

The egg-cylinder presented in figure 29 constitutes the final mesoderm-free stage, the final stage in which no distinct bilaterality may be determined. I assume that the egg-cylinder presented in the figure is cut in the sagittal plane. This assumption is based on the fact that the primary embryonic ectoderm extends slightly farther toward the mesometrial pole on the one side than on the other. In good frontal sections one side of the egg-cylinder in this stage of development should present a mirror picture of the other side. The side on which the primary embryonic ectoderm extends farther toward the mesometrial pole, the left in the figure, is regarded as containing the caudal end of the future embryo. In the primary embryonic ectoderm of this region, it is believed, will develop the primitive streak and groove, and thus the anlage of the mesoderm. Not in all the egg-cylinders of this stage of development found in my series can the caudal end of the future embryonic area be postulated prior to the anlage of the mesoderm, and in cross-sections no such differentiation can be made. The proamniotic cavity of the egg-cylinder shown in figure 29 presents a regular and nearly smooth contour, not divisible into regions such as described for a similar stage for the mouse by Selenka, Melissinos, and Sobotta. A very slight constriction is to be observed only in the region where the primary embryonic and extraembryonic ectoderm are joined in a continuous layer. I am thus wholly in accord with Widakowich, who in describing a similar stage in one of his preparations, states: "Das war die einzige konstante, bald stärker, bald schwächer ausgeprägte Einschnürung der Proamnionhöhle," as previously quoted.

A series of figures of critical regions taken from a series of cross-sections of an egg-cylinder of a stage nearly identical with that shown in figure 29, though of a slightly smaller egg-cylinder, is given in figure 30, rat No. 42, 8 days, 16 hours, after insemination. The sections chosen for the several drawings, A to D, are from the following regions, as may be ascertained by comparison with figure 29; A, through about the middle of the ectoplacental cone; B, through the proamniotic cavity just below its mesometrial end; C, through the proamniotic cavity just above the

region of the junction of the primary embryonic and extraembryonic ectoderm; D, a little above the middle of the antimesometrial portion of the proamniotic cavity. The levels of the

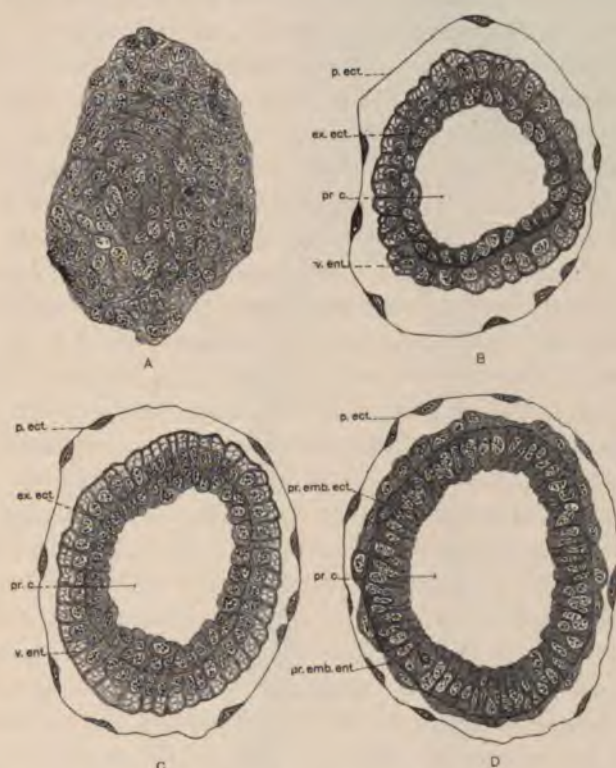


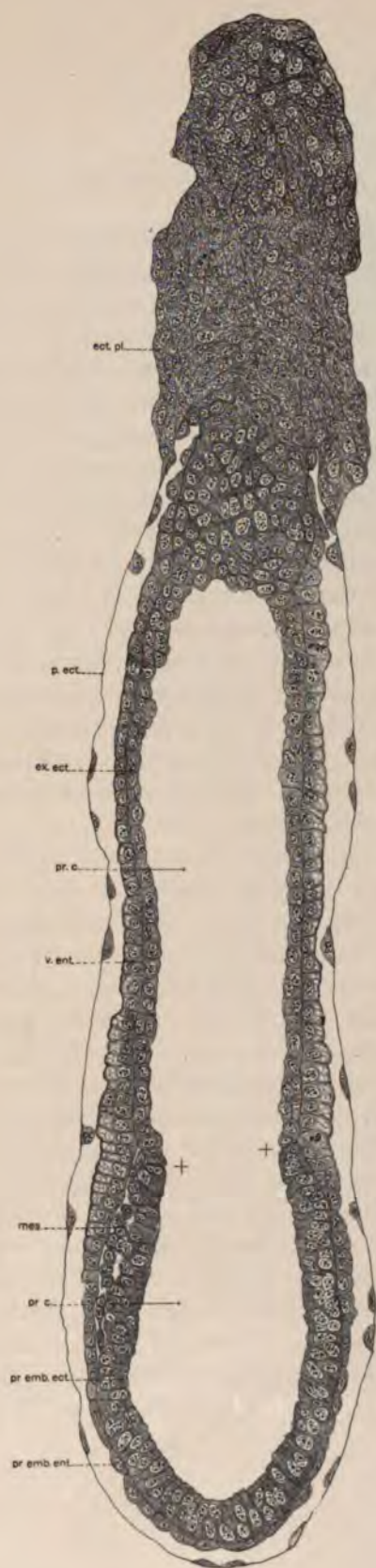
Fig. 30 Four figures from a series of cross sections of an egg-cylinder of the albino rat in the stage of development shown in figure 29. $\times 200$. Rat No. 42, 8 days, 16 hours after insemination.

The levels at which the several sections drawn were taken is approximately indicated by the several crosses found to the left of figure 29. A, middle of ectoplacental cone; B, ectoplacental end of the proamniotic cavity; C, just above level of junction of the primary embryonic and extraembryonic ectoderm; a little above the middle of primary embryonic ectoderm. The want of any definite bilateral symmetry of albino rat egg-cylinders of this stage of development is shown by this series of sections; *p.ect.*, parietal or transitory ectoderm; *ex.ect.*, extraembryonic ectoderm, surrounding mesometrial portion of proamniotic cavity; *pr.emb.ect.*, primary embryonic ectoderm; *v.ent.*, visceral entoderm; *p.emb.ent.*, primary embryonic entoderm; *pr.e.*, proamniotic cavity.

several sections drawn in figure 30 is approximately indicated by the several crosses found to the left of the egg cylinder drawn in figure 29.

In A of figure 30, there may be observed a vacuolization of the protoplasm of the more peripherally placed cells of the ectoplacental cone, the vacuoles enclosing maternal blood cells. The more centrally placed cells of this ectoplacental cone show a tendency to concentric arrangement. Figures B and C present structural appearances nearly identical. The egg-cylinder is bounded by the thin layer of parietal or transitory ectoderm having scattered masses of nucleated protoplasm on its inner surface. This membrane of apparently homogeneous structure stains sharply in well fixed preparations and may be readily discerned. The cells of the visceral entoderm, somewhat taller in the section taken nearer the antimesometrial pole (C), present clearly the three zones to which attention has been drawn. The cells of the extraembryonic ectoderm bounding the mesometrial portion of the proamniotic cavity, are of cubic, short columnar, or polyhedral form disposed in single or double layer, presenting relatively lightly staining protoplasm. In D of figure 30, the cells forming the primary embryonic ectoderm are of distinct columnar shape, with relatively deeply staining protoplasm and nuclei arranged nearly in a single layer except for such as show mitotic phases. The cells of the primary embryonic entoderm are of a broad, pavement type for a greater part of the circumference, and may be contrasted with the cells of the visceral entoderm shown in B and C of the figure; the latter are absorptive cells, the former not. This series of figures, more especially B, C, and D, show clearly the absence of bilaterality in the egg-cylinders of the albino rat at this stage of development. The slight compression observed in this egg-cylinder, as shown in the figures, I regard as not of moment.

Fig. 31 Longitudinal sagittal section of egg-cylinder of the albino rat showing anlage of the mesoderm. $\times 200$. Rat No. 34, 8 days, 18 hours, after insemination; *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *pr.emb.ect.*, primary embryonic ectoderm; *ex.ect.*, extraembryonic ectoderm; *pr.emb.ent.*, primary embryonic entoderm; *mes.*, mesoderm in anlage; *pr.c.*, proamniotic cavity; *v.ent.*, visceral entoderm.



Grosser has figured in his figures 68 and 114, an egg-cylinder of the albino rat which measures nearly 2 mm. in length. The age of this is given as $8\frac{1}{2}$ days. So far as may be determined from his figures, the preparation is not described in his text, the age, size, form, and structure of the egg cylinder shown in figure 29 and Grosser's figures 68 and 114, are very similar. In Grosser's figures, I see no evidence of his having differentiated between primary embryonic and extraembryonic ectoderm, while the reference letters for ectoderm and entoderm are reversed. Selenka's figure 31, plate 45, may be of a similar stage. This figure is, however, too diagrammatic to admit of close study. No difference is shown in the shape and structure of the cells bounding the two parts of the proamniotic cavity. Christiani's figure 39 may be of the same stage, but is too schematically drawn. Figure 4 of the article of Widakowich is of a slightly older stage and presents only a part of the egg-cylinder; it is recorded as about $6\frac{3}{4}$ days old. The stage under consideration is not figured by Widakowich, although his text description corresponds closely with what has been here presented.

The next stage and the one with which this communication is to be completed is one of importance since it is characterized by the anlage of the mesoderm. My own observations may be introduced with the consideration of an egg-cylinder, a section of which is presented in figure 31, rat No. 34, 8 days, 17 hours, after insemination. This was cut in the sagittal plane and measures 1.1 mm. by 0.2 mm., of which 0.4 mm. fall to the ectoplacental cone. This egg-cylinder is almost an exact duplicate, both in size and form, of that figured in figure 29 of the same age. In the egg-cylinder shown in figure 31, however, there may be observed, to one side, in the region of the junction of the primary embryonic and extraembryonic ectoderm, and between primary embryonic ectoderm and entoderm, a small group of cells which lie in close relation to the ectoderm and constitute early mesodermal cells. The sections of this series pass not exactly parallel to the mid-sagittal plane throughout the whole extent of the egg-cylinder; especially is this true of its antimesometrial portion, in the region of the primary embryonic

ectoderm. This portion in the section figured, passes a little to one side of the mid-sagittal plane. The two sections preceding the one figured enclose the mid-sagittal plane, and in them, the group of cells found between primary embryonic ectoderm and entoderm are in closer relation to the ectodermal layer and at all points distinctly separated from the entoderm. They are regarded as having wandered from the primary embryonic ectoderm to the place they occupy, a fact which is more easily ascertained in cross sections of a similar stage, as will appear from further discussion. From a study of very slightly older stages it can be determined that this region constitutes the primitive streak region of the future embryonic area. It is not my purpose at this time and in this communication to give especial consideration to the much discussed question of the origin of the mesoderm in Mammalia. In the rat, this question is complicated by the question of the anlage of the amniotic fold, which separates the proamniotic cavity into amniotic cavity proper and the ectoplacental cavity, the development of which will be considered in a projected contribution. In anticipation of this second publication, however, the following facts may here receive consideration. Widakowich presents in his figure 4, giving only the antimesometrial end of an egg-cylinder obtained the latter part of the 7th day, the anlage of the mesoderm as observed by him. This figure and my own figure 31 present almost identical relations, his figure showing only three mesodermal cells between primary embryonic ectoderm and entoderm. His own words concerning the anlage of the mesoderm in the albino rat, with which I find myself in full accord, except as to the age of the egg-cylinder, read as follows:

Das erste auftreten des Mesoderms beobachtete ich an Keimen vom Ende des 7 Tages. Die ersten Mesodermzellen liegen im Bereiche der vom mesometralen Ende des stärker färbbaren primären embryonalen Ectoderm gebildeten Falte. Es kommt hier eine ganz bestimmte Stelle in Betracht, die dort liegt, wo sich später das hintere Ende des Primitivstreifens befindet.

There is, however, wide divergence of the views of authors as concerns the anlage of the mesoderm in the rat and mouse.

Selenka, it would seem, in part at least, interpreted correctly the development of the mesoderm in the rat, although a stage showing its anlage was not observed. Duval believes that the mesoderm has origin from a thickened part of the entoderm, probably in the region of the anterior portion of the future embryonic area; the primitive streak was not recognized. Christiani's figures 45 and 47, transverse sections of the egg-cylinder from the eighth day, give correctly the relative position of the mesoderm with reference to the primitive streak; however, they show stages some little time after the anlage of the mesoderm. According to Robinson, in the early part of the eighth day the cavities of the epiblast (primary embryonic ectoderm) and of the trophoblast (extraembryonic ectoderm) meet and fuse to form a hollow cylinder, the proamniotic cavity. He states that "For a time the united cavities of the epiblast and trophoblast increase in size, together with the general growth of the ovum, and this increase continues until in the latter part of the eighth day the mesoblast appears around the margin of the epiblast where it is in apposition with the trophoblast." Robinson was able to differentiate between the primary embryonic ectoderm (epiblast) and the extraembryonic ectoderm (trophoblast) and his figure 14 (plate 23-24), though schematic, shows that he recognized the positions of the anlage of the mesoderm correctly, as also its derivation from the primary embryonic ectoderm. The observations of Melissinos, bearing on the anlage of the mesoderm have been critically reviewed by both Widakowich and Sobotta, and I am wholly in accord with their views when they state that no credence can be given these observations since it is clear that Melissinos has confused sagittal and frontal sections in such a way as to make his observations of no value. According to Melissinos, the mesoderm arises from the outer surface of the middle fold of the egg cylinder, in the region of its union with the antimesometrial ectodermal fold; it is certain that it does not arise from the part of the egg-cylinder that has differentiated from the primary embryonic ectoderm; but, if I interpret him correctly, from the extraembryonic portion of the ectoderm. That Melissinos did not

have before him the stages showing the anlage of the mesoderm seems clear. Sobotta's ('11) observations, mouse material, deserve fuller consideration. In interpreting his results, I am mindful of the fact that he was unable to locate the line of union between primary embryonic and extraembryonic ectoderm, as can readily be done in suitable rat material, as has previously been shown by Robinson and Widakowich, and to which attention has constantly been drawn in this communication. I am unable to state from personal observation whether in the white mouse these two types of ectoderm which form the lining of the proamniotic cavity, can be differentiated on ascertaining the right technical method. Sobotta's material seems well fixed. If not, it would seem to me difficult to determine definitely the exact place of origin of the mesodermal cells, whether extraembryonic or embryonic. Sobotta recognized the anlage of the mesoderm in the mouse during the last hours of the seventh day or first hours of the eighth day. This is said to appear at the caudal end of the future embryo as a group of loosely arranged cells lying between the inner and outer layers of the egg-cylinder. At the place where the mesodermal cells arise from the inner layer of the egg-cylinder, there is developed a fold, recognized as the caudal amniotic fold ("Schwanzfalte des Amnios"). After discussing these observations at length, Sobotta concludes as follows:

Was die Deutung dieser frühen Stadien der Mesodermbildung in der Keimblase der Maus anlangt, so handelt es sich hier nicht um die Bildung des embryonalen Mesoderms, die erst mit der eigentlichen Gastrulation später einsetzt, sondern um Entstehung ausserembryonalen Mesoderms, besonder des Teils des mittleren Keimblattes, dass bei der Bildung der primären Eihäute, Amnios und Chorion in Betracht kommt und des den ausserembryonalen Teil der Leibeshöhle, das Exocoelom auskledet, der Höhle, die eben Amnios und Chorion voneinander trennt. Es erfolgt also, um einen kurzen Ausdruck zu gebrauchen, die Bildung des Amniosmesoderms.

An embryonic anlage is said not to exist at this stage; this is recognized only after the development of the primitive streak. It is not my purpose to enter fully into a discussion of this important question in this communication. This would involve

consideration of older stages, and the making of a number of reconstructions, which it is not contemplated to consider now. It must suffice to state at this time that in the albino rat, as shown by Widakowich and here shown by me, it is possible to delineate clearly the primary embryonic ectoderm and to show that the first evidence of the mesoderm is found antimesometrial to the future amniotic fold and in the region of the future primitive streak; therefore is mesoderm which I would regard as peristomal mesoderm in the sense of C. Rabl, reference to which is made by Sobotta in his discussion of this question. It may be that the rat offers more suitable material for the elucidation of this question than is to be found in the mouse. In the albino rat, the anlage of the mesoderm is from the sagittal portion of the caudal region of the primary embryonic ectoderm, the caudal part of the future primitive streak and antimesometrial to the amniotic fold. Sobotta gives very favorable consideration to the observations of Widakowich, touching this question, which he regards as "Bei weitem die beste Darstellung des Gegenstandes." My own observations fully confirm those of Widakowich. These questions will receive fuller consideration in a later publication dealing with the embryology of the albino rat, carrying the development from the time of the anlage of the amniotic fold to the stage of embryo form, the material for which is at hand.

In figure 32 are shown cross-sections of the antimesometrial portion of three egg-cylinders in the region of the developing mesoderm. Sections drawn in A and B, were taken respectively from egg-cylinders obtained from the same uterus as was the one shown in sagittal section in figure 31, rat No. 34, 8 days, 17 hours, after insemination; C, from rat No. 41, 8 days, 16 hours, after insemination. It is very probable that the series from which A of this figure was drawn, is not cut in exactly the cross plane. A study of the series shows, however, that the deviation from this plane is not marked. The sections from which this figure was drawn pass a little below (antimesometrial) to the region of junction of the primary embryonic and extraembryonic ectoderm. To one side, the lower in the figure, the primary embryonic ectoderm shows a slight thickening and evidence of

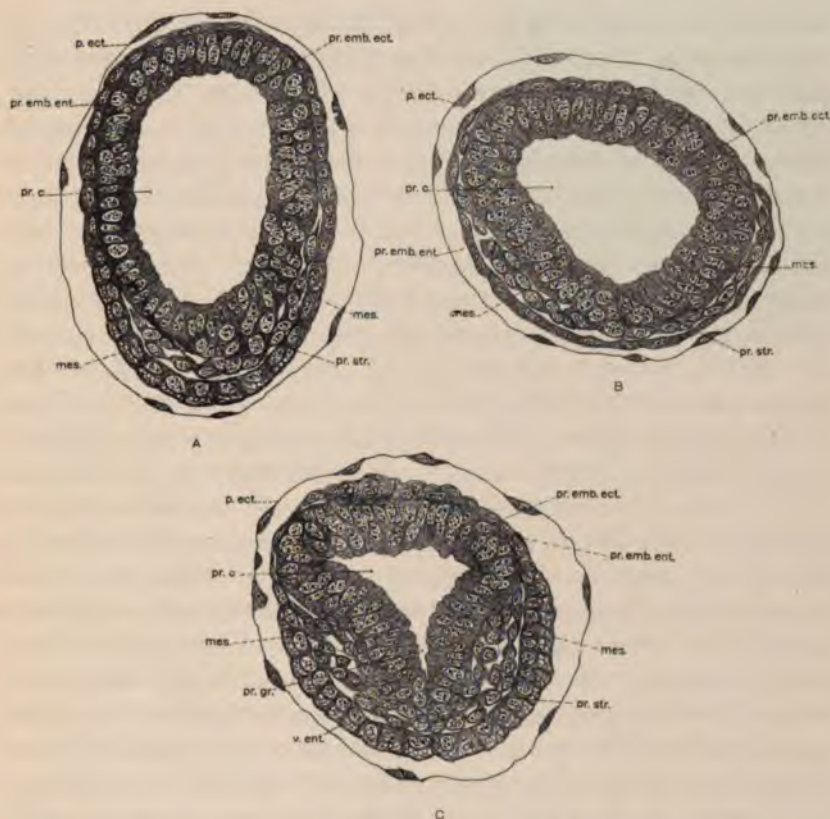


Fig. 32 Three cross sections from egg-cylinders of the albino rat, showing early stages in the development of the mesoderm. $\times 200$. A and B, rat No. 34, 8 days, 17 hours; C, rat No. 41, 8 days, 16 hours, after insemination.

These sections taken from three egg-cylinders are through the primary embryonic ectoderm, near its junction with the extraembryonic ectoderm, thus through the antimesometrial portion of the proamniotic cavity. A, early stage, in anlage of the mesoderm; B, anlage of the primitive streak and groove; C, well developed primitive streak and groove, with lateral wings of mesoderm; *pr.emb.ect.*, primary embryonic ectoderm; *pr.emb.ent.*, primary embryonic entoderm; *mes.*, mesoderm; *pr.str.*, primitive streak; *pr.gr.*, primitive groove; *p.ect.*, parietal or transitory ectoderm; *pr.c.*, proamniotic cavity.

cell proliferation. The cells of this region have not the form of tall columnar cells, such as seen in the greater part of the remaining primary embryonic ectoderm, but are of polyhedral

form and are continuous, in the mid sagittal plane, with cells that have wandered between the primary embryonic ectoderm and entoderm, cells regarded as constituting the mesoderm. In all of the sections of this series, so far as the mesoderm extends, this is distinctly separable from the entoderm, and is continuous with the primary embryonic ectoderm only along a narrow region of thickened primary embryonic ectoderm, situated in the mid-sagittal plane, and which may in this series be regarded as the anlage of the primitive streak. From the sides of this region of slightly thickened primary embryonic ectoderm, the extent of which is evidenced by the absence of an external limiting membrane, cells wander laterally to form the mesoderm. B, of figure 32, presents essentially the same appearance, although representing a slightly older stage. The sections of this series I regard as cut fairly well in a plane at right angles to the long axis of the respective egg-cylinder. The section taken for the sketch is situated a very little further away from the line of junction of the primary embryonic and extraembryonic ectoderm, than is the section the drawing of which is shown in A of this figure, as may be judged from the more uniformly pavement type of the entodermal cells. The triangular form of the proamniotic cavity is regarded as normal, and as indicating an early stage in the anlage of the primitive groove. In this figure, in its lower portion, the region of the primitive streak is readily discernible by reason of the fact that there is wanting here an external limiting membrane, and further by reason of the form of the cells and the form and relative position of their nuclei; certain of these cells indicating, both by their form and their position, the source and the direction of the wandering of the cells which constitute the anlage of the mesoderm. The wandering of the mesodermal cells between the primary embryonic ectoderm and entoderm, to form the lateral mesodermal wings, is clearly shown in this figure, especially to the left. The anti-mesometrial ends of the egg-cylinders, sections of which are shown in A and B of this figure, are as yet free from the invading mesoderm, as is also the part of the egg-cylinders lying opposite the region of the primitive streak, the upper portions of the

respective figures, these forming the region of the future anterior ends of the respective embryos. In C of figure 32 is shown a drawing of one of the sections of a series of cross-sections of an egg-cylinder taken from rat No. 41, 8 days, 16 hours, after insemination, presenting a stage in which the primitive groove may be definitely made out. This figure is not unlike figure 6 of the article of Widakowich, obtained from an egg-cylinder secured on the eighth day. Concerning this figure he states: "Das Ectoderm steht in direktem Zusammenhange mit zwei Mesodermzungen die gegen die der Primitivrinne gegenüberliegende Seite zu auswachsen." The section drawn in C of this figure is taken from the region very near the junction of the primary embryonic and the extraembryonic ectoderm, as may be observed from the character of the entodermal cells, in the lower part of the figure. The increase in the thickness of the mesodermal wings, the result, in part at least, of proliferation of mesodermal cells, as evidenced by the presence of mitotic figures, is clearly shown in this figure. The mesoderm is distinctly separable from the entoderm as also from the primary embryonic ectoderm except in the region of the primitive streak and groove. The growth of the mesoderm after its anlage has been correctly shown for the albino rat by Slenka, Robinson, and Widakowich; the latter especially giving excellent figures. His figure 5 is especially instructive. In this, he represents the appearances shown by two views of an isolated egg-cylinder, with the primitive groove in anlage, showing the lateral extensions of the mesoderm. Sobotta ('11) has given the best and most comprehensive account of the anlage and growth of the mesoderm in the mouse. An excellent cross-section of a mouse egg-cylinder in the primitive streak stage is presented in his figure 6, which presents very similar appearances to my C of figure 32. None of the figures of cross-sections of egg-cylinders included by me show the very beginning of the anlage of the mesoderm, though A of figure 32 approaches this very closely, as does also figure 31, presenting a sagittal section. The evidence at hand warrants the conclusion that in the albino rat, the mesoderm has its anlage in the caudal region of the primary embryonic

ectoderm, from a narrow zone of cells situated in the region of the future primitive streak. From this region there is an outwandering of cells which invade the potential cleft between primary embryonic ectoderm and entoderm, spreading laterally in wing-like sheets. This I would regard as prostomial mesoderm in the sense of C. Rabl. The anlage of the mesoderm in the albino rat, and the early stages of its lateral extension, with the anlage of the primitive streak and groove, falls to the latter part of the ninth day after insemination.

Beginning with the pronuclear stage, found at the end of the first day, 8 days are required for the completion of the process of segmentation, blastodermic vesicle formation and the formation of the primary germ layers—ectoderm, mesoderm, and entoderm—in all, 9 days out of a possible 21 to 23 days, the normal gestation period of the albino rat.

(CONCLUSIONS)

Early stages of mammalian development may readily be obtained from the albino rat (*Mus norvegicus albinus*). When care is exercised, mating may be observed and the age of the embryo, reckoned from the time of mating (insemination), determined with a fair degree of accuracy. Ovulations occur about the time of parturition and again 29 to 30 days post partem. This latter period is more favorable for obtaining insemination and semination, thus fertilized ova. The process of fertilization probably takes place during the latter half of the first day after insemination.

The pronuclear stage, a stage which extends through a period of perhaps 12 to 15 hours, in the middle phase, is observed at the end of the first day after insemination; the fertilized ova having wandered about one-fourth of the length of the oviduct by that time. Of the two pronuclei, the female pronucleus is slightly the larger. The two pronuclei lie near the center of the ovum, are distinctly membraned, and do not fuse prior to the formation of the first segmentation spindle.

The formation of the first segmentation spindle and the first segmentation occur during the early part of the second day after

insemination. The resulting 2-cell stage extends for a period of about 24 hours and is found in about the middle of the oviduct. The first two blastomeres are equivalent cells. One of these segments before the other, resulting in a 3-cell stage, present for each ovum for only a relatively short period.

The 4-cell stage is observed at the end of the third day after insemination. The ova have by this time traversed about nine-tenths of the length of the oviduct.

The 8-cell stage is observed the latter half of the fourth day after insemination and at the end of the fourth day the ova pass from the oviduct to the uterus in the 12-cell to 16-cell stage. The oolemma is lost usually in the 4-cell stage, the segmenting ova conforming in shape to the general form of that portion of the oviduct in which they are found.

Three successive segmentation stages, spaced at intervals of about 18 hours, resulting in 2-, 4-, and 8-cell stages occur during transit through the oviduct. During the fourth segmentation the ova pass from the oviducts to the uterine horns, at the end of the fourth day.

The mass increase of the ova during the first three segmentations is approximately from 0.15 c.mm. in the pronuclear stage to 0.18 c.mm. in the 8-cell stage. The slow rate of segmentation and the relatively small mass increase may be attributed to the relative scarcity of the embryotroph during transit through the oviducts.

During the early hours of the fifth day after insemination, all of the segmenting ova are found lying free in the lumen of the uterus, spaced about as in the later stages of development, the fifth series of segmentations having been completed by this time, the resulting morula masses having ovoid form, measuring approximately $80\ \mu$ by $50\ \mu$ and consisting of from 24 to 32 cells. The mechanism operative in spacing the ova in the uterine horns has not been determined.

The early stages of blastodermic vesicle formation are observed during the middle and latter half of the fifth day. The segmentation cavity begins as a single, irregularly crescentic space, eccentric in position, and arising between the cells of the morula.

By the end of the fifth day after insemination, all fertilized, normal ova are found in the blastodermic vesicle stage. One pole of each vesicle, its floor, consists of a relatively thick mass of cells, in which there is no differentiation in layers and no evidence of ectodermal and entodermal cells. The other pole of each vesicle, its roof, consists of a single layer of flattened cells, bordering the segmentation cavity.

During the sixth day, the blastodermic vesicles which still lie free in the lumen of the uterus, increase in size, partly as a result of extension of the roof cells, partly owing to rearrangement and flattening of the cells of the floor. This portion of the vesicle now presents the form of a concavo-convex disc, forming about one-sixth of the vesicle wall and consisting, as a rule, of three layers of cells, the inner of which is now differentiated to form the yolk entoderm.

During the seventh day after insemination the blastodermic vesicles become definitely oriented in a decidual crypt, the thicker portion, its floor, being directed toward the mesometrial border. The phenomenon of the "inversion of the germ layers" or "entypy of the germ layers" is initiated, the result of cell rearrangement and cell enlargement in the germinal disc, manifested as an outgrowth to form the ectoplacental cone or Träger and an ingrowth into the vesicle, the anlage of the egg-plug or egg-cylinder. In the egg-plug there is recognized a circumscribed, compact mass of cells, staining more deeply than surrounding cells, which constitute the ectodermal node, the anlage of the primary embryonic ectoderm of the future embryo. This ectodermal node, so far as it extends into the cavity of the blastodermic vesicle, is surrounded by yolk entoderm.

During the eighth day after insemination, the egg-cylinder comes in definite relation with the maternal decidua and receives as embryotroph maternal hemoglobin, partly through phagocytic action of the cells of the ectoplacental cone, partly through absorption of maternal hemoglobin by the cells of the entoderm, initiating a period of very active growth as evidenced by active mitosis. The egg-cylinder increases in length, and entypy is completed. A cavity develops in the ectodermal

node, the antimesometrial portion of the proamniotic cavity. A little later a second cavity develops in the extraembryonic ectoderm, the mesometrial portion of the proamniotic cavity, the two cavities fusing by the end of the eighth day to form a single proamniotic cavity, lined in its antimesometrial portion by primary embryonic ectoderm, and in its mesometrial portion by extraembryonic ectoderm, the two types of ectoderm forming a continuous layer with the line of junction readily distinguishable. No evidence of bilateral symmetry is at this stage observed in the egg-cylinder.

During the ninth day after insemination there is observed the anlage and the early developmental stage of the mesoderm and the anlage of the primitive streak and groove. The mesoderm has its anlage in the caudal portion of the primary embryonic ectoderm in the sagittal region and is of the nature of prostomial mesoderm, extending laterally in wing-like extensions between the ectoderm and entoderm.

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THE DEVELOPMENT OF THE ALBINO RAT,
MUS NORVEGICUS ALBINUS

II. ABNORMAL OVA; END OF THE FIRST TO THE END OF THE
NINTH DAY

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INTRODUCTION

In the course of my study of the normal development of the albino rat, from the end of the first to the end of the ninth day after insemination, as recorded in Part I of this series of contributions, there were encountered from time to time ova which appeared to deviate both in rate and type of development from what, as a result of extended study, came to be regarded as the normal developmental cycle of the albino rat. When taken collectively, the number of these abnormal ova is not large, although they embrace nearly all of the developmental stages studied. When taken singly, it may be stated that while it is comparatively easy to record the points of deviation from the normal, it must be admitted that the probable fate of the respective stages can only be conjectured. Nevertheless, a record of

the abnormal stages met with seems warranted, especially in view of the fact that the literature is very meager in its account of early stages of mammalian ova presenting abnormal development.

The excellent and comprehensive studies of Mall on pathologic human ova, extending over many years, may be interpreted as leading to the general conclusion that pathologic ova and monsters "are produced from normal eggs by conditions which either interfere with their nutrition or poison them." There is evidence to show that defective implantation, using the term in its broadest sense so as to include relation to the embryotroph or pabulum, is directly associated with abnormal development. Comparative experimental teratology so successfully followed by a number of European and American experimental embryologists warrants the conclusion that all of the abnormalities or malformations observed in the human embryo may be brought forth by the application of suitable mechanical interference or chemical solutions. Experimental teratology possesses the very great advantage of enabling the observer to follow the pathologic process from step to step, admitting more readily of their interpretation, than when single stages are obtained from nature. The evidence appears to be accumulating that the primary causes which produce pathologic ova lie not in the germ cells, but are rather to be sought in the environs of the germ cells in the course of their development.

I am cognizant of the fact that the interpretation of the chance findings of abnormal stages of mammalian ova is much more difficult than of abnormal ova produced experimentally. The fact, however, that nearly all of the abnormal ova observed by me in my albino rat material were found in tubes and uteri containing normal ova also, tubes and uteri which so far as observable appear in most instances to be normal, and the further fact that certain of the abnormal ova are of stages prior to what may be regarded as showing implantation, stages concerning which we possess no data as far as human ova are concerned, has led to the tentative conclusion that certain of the abnormal ova may be the resultant of abnormal germ cells, perhaps of an abnormality which may not show a structural expression.

It is my primary purpose to make records of the abnormal ova observed in the material at hand; and to follow these records with a brief consideration of the observations made. There is no literature dealing with the problem immediately at hand—abnormal rat ova. It is not my purpose at this time to enter into the extensive literature of comparative experimental teratology. This has been critically summarized relatively recently by O. and R. Hertwig, and by Mall, in his several contributions dealing with human pathologic ova.

HALF EMBRYOS IN MAMMALIA

The first preparation to which attention is called is one taken from the oviduct of rat No. 60, 1 day, 18 hours, after insemination. The two oviducts of this rat contained seven ova in the 2-cell stage, to one of which especial attention was drawn in Part I (page 271). As there recorded, in one of the 2-cell stages, the first two blastomeres were separated by an appreciable distance. There is loss of oolemma. The possibility of half embryos in Mammalia was suggested. The preparation under consideration is figured in figure 1, A and B. In A of this figure there is presented a portion of the wall of the oviduct, its epithelial lining and the immediately adjacent mucosa, including the fourth of a series of six sections ($10\ \mu$) passing through the two blastomeres. In this region, the cilia of the epithelium are clearly observable, as may be seen from the figure. In B of this figure there are sketched in approximately relative position the several sections of the series passing through the two blastomeres, the relative position of which, with reference to the walls of the tube, is shown in A of the figure. The six drawings were made from a well ribboned series; the slide was moved from section to section by means of a mechanical stage, and the perpendicular indicated on each drawing as made. The relative position of the several drawings, therefore, is quite correct. It may be observed that throughout the series the two blastomeres are separated by an appreciable space, and that one of the cells has rotated slightly on its axis. If these two blastomeres had remained in close apposition, they would present the appearance of a normal 2-cell stage



Fig 1 Oviduct and ovum of albino rat, in 2-cell stage, with first two blastomeres separated. Rat No. 60, 1 day, 18 hours, after the beginning of insemination. $\times 200$. A, epithelial wall of oviduct with adjacent mucosa, and the fourth of a series of six sections of the 2-cell stage with separated blastomeres, showing them in their relation to the epithelium. B, the series of six sections which pass through the separated blastomeres, the fourth of which is shown in A. The series reads from right to left.

as shown in B and C of figure 1, Part I. There is here clearly a separation of the first two blastomeres and not a close approximation of two unfertilized ova. In all of the unfertilized ova met with in the oviducts in the series at my disposal, these present the second maturation spindle and oolemma and are not to be confused with the blastomeres of the 2-cell stage, either as to size or structure. Both of the blastomeres in the preparation under consideration present normal protoplasmic structure, having a finely granular protoplasm. Their nuclei, as may be seen from the figures, are of normal size and structure. They present regular form, are distinctly membranated, have large chromatoid nucleoli, and chromatin scattered in fine granules and threads. However, attention needs to be drawn to the presence of two micro-nuclei, one in each of the two blastomeres, showing in the third and fourth section of the series respectively (B, fig. 1). These micro-nuclei are nearly free from chromatin, each presenting a small chromatoid nucleolus. They are not to be regarded as cell inclusions, as perhaps representing phagocytic leucocytes. It may be conjectured that they were formed by amitotic division, by budding and constriction from the parent

nuclei, perhaps indicating altered metabolism in the two blastomeres. I am inclined to think that both of these cells would have degenerated in the course of further development; however, their fate can only be guessed and not predicted. The possibility of their developing into half embryos is suggested. Half embryos developing as a result of a separation of the first two blastomeres has not been observed in the Mammalia, and an experimental test of the question is for the present not a probability.

As a result of experimental embryology it has been clearly shown that through mechanical interference polysomatous monsters may be produced from normal ova. The first two blastomeres are totipotent, as expressed by Driesch. Driesch was able to produce polysomatous forms by mechanical separation of the first two blastomeres in sea urchin eggs; Wilson, by separating through shaking of 2- and 4-cell stages in *Amphioxus*; O. Hertwig, Herlitzka and Spemann, by separating the first two cells in amphibian eggs; O. Schultze and others, by use of gravity and compression; and Loeb and others by use of chemical agents. By various means, then, when suitably applied and at the right time, hemiembryos have been produced by separating or potentially separating the first two blastomeres in certain forms. O. Hertwig states:

Bei den kleinen, mit geringen Mengen von Dotter ausgestatteten Eiern der Wirbeltiere sind spontan entstandene, das heisst, ohne experimentelle Eingriffe veranlasste Mehrfachbildungen ausserordentlich selten, bei manchen Klassen überhaupt noch nie beobachtet worden, dagegen sind sie relativ häufige Befunde bei manchen untersuchten Arten von Knochenfischen und Vögeln, besonders bei der Forelle und beim Hühnchen.

So far as I am aware, the possibility of hemiembryos in Mammalia has not been shown. In the albino rat, the oolemma may be lost as early as the 2-cell stage. In forms with early loss of oolemma, the separation of the two first blastomeres does not appear to me as an impossibility. The probable fate of separated mammalian blastomeres can only be conjectured, since it is manifestly impossible, for the present, to follow them in further development.

*

DEGENERATION AND DEATH OF OVA AT THE END OF
THE SEGMENTATION STAGES

In figure 2, A and B, are presented drawings of typical sections of two morula masses showing complete degeneration and death. The degenerated ovum shown in A, of this figure was obtained from rat No. 52, 4 days, 15 hours, after insemination. In all, eight normal ova were found in the uterus of this rat,



Fig 2 Ova of the albino rat in late segmentation stages, showing death and dissolution of the constituent cells. $\times 200$. A, rat No. 52, 4 days, 15 hours, after the beginning of insemination. B, rat No. 68, 4 days, 16 hours, after the beginning of the insemination. This figure shows an imperfectly developed morula with probable retention of oolemma.

these showing late morula stages and stages of early blastodermic vesicle formation, three of which were sketched and are shown in A, B, and C of figure 20, Part I. The degenerated ovum here under consideration lies in very close proximity to the normal blastodermic vesicle shown in C of figure 20, Part I. The shallow mucosal pits harboring the two ova are in contiguity. The two contiguous pits resemble each other very much; the mucosa underlying them is in every respect the same, indicating, it would seem, that to a certain stage in development—to the end of segmentation—the development of the degenerated ovum proceeded normally. The degenerated egg-mass measured approximately $80\ \mu$ by $50\ \mu$ by $40\ \mu$. In reaction to stains, it differs markedly from the adjacent normal vesicle. The staining is very pale; cell boundaries are indistinct or lost, and the nuclei scarcely retain any coloring matter. Scattered through the protoplasm are found small globular masses, perhaps of lipoid character. Protoplasm and nuclei present evidences of cytolysis and chromatolysis, and have the appearance presented by necrotic tissue. Had normal development supervened, both ova

(the pathologic and the adjacent normal one) would in all probability have been enclosed within the same decidual crypt, a condition exceedingly rare, judging from the material at hand. Whether the very close proximity of these two ova bears causal relation to the death of one, by reason of the consequent lessening of the available pabulum or embryotroph, can only be conjectured. There is at this stage no question of faulty implantation, the ova, though presumably permanently lodged, lie free in the lumen of the uterus. Whether on the other hand, the death of this ovum was the result of some inherent nutritional deficit must also remain unanswered. However, this preparation may serve to show that ova of the albino rat, after reaching the uterine tube, and after apparently normal segmentation, may undergo death and dissolution, for reasons which are not structurally discernable.

B of figure 2, rat No. 68, 4 days, 16 hours, after insemination, is from the uterus of a rat containing four ova in early stages of blastodermic vesicle formation, three of which were sketched under D and E of figure 20, and the series of figure 21, Part I. The preparation here described lies free in the lumen of the uterus, and appears to represent an uncompleted segmentation, with cells and nuclei showing cytolysis and chromatolysis. The mass is surrounded by a thin membrane regarded as an oolemma. Normally the oolemma of the segmenting ova of the albino rat is lost in the 4-cell stage, now and again in the 2-cell stage. Whether the retention of the oolemma may be brought in causal relation to the death and dissolution of the enclosed cells is problematic. That such causal relation may exist for the ova of the albino rat, appears to me as not impossible. This degenerated egg-mass presents the only instance of the late retention of the oolemma in the albino rat material at my disposal.

INCOMPLETE OR RETARDED SEGMENTATION

The blastodermic vesicles presented in figures 3 and 4 have been interpreted as showing incomplete or retarded division of certain of the cells of early stage morula masses. The probable fate of such blastodermic vesicles in further development cannot

be projected with any degree of certainty. The most characteristic vesicle showing this phenomenon is presented in figure 3, and is taken from rat No. 53, 5 days after insemination, the uterus of which contained seven blastodermic vesicles showing early stages of development, four of which are reproduced in figure 22, Part I. In A and B of figure 3 are reproduced two consecutive sections of a series of five sections of $10\ \mu$ thickness, including this ovum. In the lower part of this ovum there is found a small segmentation cavity, bounded by cells which present normal appearances. The roof of this vesicle is slightly



Fig. 3 Early stages of the blastodermic vesicle of the albino rat, presenting evidence of irregular or retarded segmentation. $\times 200$. Rat No. 53, 5 days after the beginning of insemination.

Fig. 4 Three ova of the albino rat, showing early blastodermic vesicle stages, in each of which certain of the cells suggest irregular or retarded segmentation. $\times 200$. A, rat No. 64, 4 days, 14 hours, after the beginning of insemination. B, rat No. 68, 4 days, 15 hours, after the beginning of insemination. C, rat No. 54, 6 days, 16 hours, after the beginning of insemination.

folded and compressed, as a consequence of which the roof wall in the sections figured is presented in part as seen in surface view. In the floor of this vesicle there is to be observed, surrounded by other smaller cells, one large cell, of nearly spherical shape, having a diameter which is three or four times as great as that of the majority of the surrounding cells. The protoplasm of this large cell stains less deeply than does that of the majority of the other cells constituting the floor of the vesicle. Its nucleus is relatively large and slightly lobulated, so much so that in the section of it shown in A of this figure, in the optical section sketched, the nucleus appears as three separate nuclei, in reality,

lobules of the same nucleus. In A of this figure there is shown to the lower left of the large cell another relatively large cell, enclosing a globular inclusion, which stained faintly, and the nature of which was not fully determined. In the upper part of each of the two figures are seen cells which show cytolysis and loss of nuclei; regarded as degenerating cells. When compared with the normal blastodermic vesicles obtained from the same uterus, the ovum here described presents a unique appearance, and was readily recognized as showing development and structure which deviated from the normal. At this stage of development, the blastodermic vesicles of the albino rat are still found lying free in the lumen of the uterus, showing no structural relation to the uterine mucosa. This vesicle has been interpreted as showing irregular or retarded segmentation. It is conjectured that one of the cells, perhaps of the 8-cell stage, did not undergo further cleavage. The large cell presents an appearance evidencing beginning stages of degeneration, and in further development, would probably have undergone dissolution. The majority of the smaller cells of the roof appear as if normal, as do also the cells of the floor, certain of the smaller cells of the floor presenting mitoses as evidence of further proliferation.

In figure 4, A, B, and C, there are presented typical sections of three ova of the albino rat showing what has been regarded as irregular segmentation. A of this figure represents an ovum taken from rat No. 64, 4 days, 14 hours, after insemination, in the uterus of which there were found five normal ova showing early stages of blastodermic vesicle formation, four of which are cut longitudinally, one in a series of cross-sections. In each of the four longitudinally cut series the floor of the respective vesicles is markedly folded, owing to fixation contractions; therefore, none were sketched as normal stages. In appearance, they resemble closely the vesicles sketched under C, D, and E of figure 20, Part I. In the pathologic ovum, shown in A of figure 4, there is no evidence of segmentation cavity formation. However, the ovum cannot be regarded as presenting a late morula stage such as is figured in A of figure 20, Part I, since it shows distinct departure from the normal. The marked constriction

seen to the lower left of the figure passes through the series of four 10 μ sections including this ovum, and in part separates a portion composed of relatively small cells from a larger portion composed of larger cells. The rate of segmentation of certain of the cells composing the upper larger portion of this cell mass appears to have been retarded, thus retarding the development of the whole mass. This pathologic ovum rests normally in a shallow pit of the mucosa, very similar in form and structure to the shallow pit lodging the five normal vesicles found in this uterus.

The ovum shown in B of figure 4 was obtained from the uterus of rat No. 68, 4 days, 16 hours, after insemination. with four normal vesicles showing early stages of blastodermic vesicle formation. From this uterus was also taken the completely degenerated cell mass with persistent oolemma shown in B of figure 2. This vesicle on superficial observation does not appear to depart markedly from the normal appearance for this stage. In form and size it corresponds closely to the normal ova taken from this uterus. The segmentation cavity seems to have developed normally. The slight folding of the roof seen to the left of the figure is accidental, due to fixation shrinkage, and is very similar to folding of the roof to be observed in many of the normal preparations of the series. In the floor of the vesicle there may be observed three relatively large cells, partly enclosed by smaller cells of a size comparable to that of the cells forming the floor of the normal blastodermic vesicles of this stage of development. The three relatively large cells, clearly distinguished in the figure, are interpreted as showing a retarded segmentation. So far as may be determined, their protoplasm and nuclei present normal structure, the lowest of the three cells showing an early mitotic phase. I am inclined to the opinion that this ovum would have continued in development, perhaps in later stages showing distinct arrest in development. This hypothesis seems warranted on the basis of the study of a vesicle shown in C of figure 4, taken from rat No. 54, 6 days, 16 hours, after insemination. Normal stages for the albino rat, taken about the middle of the seventh day after insemination, are shown in figure

24, Part I. Reference to this figure may serve to show that during the early hours of the seventh day after insemination, the phenomenon of inversion or entypy of the germ layers is initiated in the albino rat. The ova are, on reaching this stage of development, enclosed within a well differentiated decidual crypt which communicates as yet freely with the lumen of the uterus. These crypts present a continuous lining of uterine epithelium; the contained ova are thus not as yet in direct relation with the maternal decidua. In the normal blastodermic vesicle of this stage, the ectoplacental cone is in anlage, and in the cell mass which extends into the cavity of the vesicle—the egg-plug or egg-cylinder—there is evident a clearly circumscribed nodule of cells, which has been designated the ectodermal node and recognized as the anlage of the primary embryonic ectoderm; this node is in part surrounded by the yolk entoderm. In the uterus of rat No. 54, there are contained nine blastodermic vesicles, one of which is sketched in C of figure 24, Part I. Not nearly all of these vesicles are so favorably cut as that shown in this figure, the majority being cut in a plane which is oblique to the long axis of the vesicle. However, in all of them the ectoplacental cone and the ectodermal node may be determined except in the one shown in C of figure 4. This vesicle was obtained from a series of sections passing at right angles to the plane of the mesometrium. It lies free in a deep decidual crypt and passes through six sections of 10 μ thickness; thus is compressed from side to side. This vesicle is distinctly smaller than the normal ones taken from this series, especially so as concerns its cavity. An ectoplacental cone is not clearly differentiated, and it is not possible to determine an ectodermal node, nor is it clear that the yolk entoderm has differentiated. In the cell mass from which ectoplacental cone and ectodermal node should have developed, the upper portion of this figure, there are evident, in the sections figured, four relatively large cells with relatively large nuclei, cells which have been interpreted as evidencing retarded segmentation with consequent retardation in the normal differentiation of the vesicle. On tracing this vesicle through the series of six sections it would seem that the direction of section is favor-

able. The uterine mucosa appears to have reacted normally; the decidual crypt in which this vesicle is lodged presenting normal size and form, and the surrounding decidua normal structure. The vesicle itself is retracted from the uterine epithelium, intact throughout the crypt, thus, does not appear to have attained the normal adhesions observed in normal vesicles of this stage. The four ova depicted in figures 3 and 4, appear to present a distinctive type of abnormal development, a type which is interpreted as showing retarded segmentation in certain of the cells of the 8-cell and perhaps 16-cell stage. All are found in



Fig. 5 Four consecutive sections of the ovum of the albino rat showing abnormal development of the segmentation cavity $\times 200$. Rat No. 46, 6 days, 14 hours, after insemination.

uteri containing normal stages. The appearances presented, if correctly interpreted, speak in favor of a structural or metabolic defect inherent in the cells themselves and not primarily dependent on environment, pabulum, or embryotroph.

ABNORMAL SEGMENTATION CAVITY FORMATION

The following three ova have been grouped as showing irregularity in the formation of the segmentation cavity.

In figure 5 are reproduced four consecutive sections passing through an abnormal ovum obtained from rat No. 46, 6 days, 14 hours, after insemination. There were obtained from the uterus

of this rat ten blastodermic vesicles, two of which are reproduced in A and B of figure 24, Part I, as showing typically early stages of the anlage of the ectoplacental cone and entypy of the germ layers. The ovum shown in figure 5 is found in a decidual crypt which is in very close proximity to the one containing the vesicle figured under B of figure 24, Part I, the two crypts being separated by a distance of approximately 1.3 mm., while the distance between decidual crypts is normally 1 cm. to 1.5 cm. The decidual crypt lodging the abnormal ovum presents a normal appearance, resembling very closely in form, depth and structure



Fig. 6 Two ova of the albino rat, interpreted as evidencing retarded or irregular formation of the segmentation cavity. $\times 200$. A, rat No. 90, 6 days, 17 hours, after the beginning of insemination. B, rat No. 90, 6 days, 17 hours, after the beginning of insemination. *p.ect.*, parietal or transitory ectoderm; *y.ent.*, yolk entoderm; *p.ent.*, parietal entoderm.

of the surrounding decidua, the crypt and decidua enclosing the adjacent normal vesicle figured in B of figure 24, Part I. The abnormal ovum in question appeared to have proceeded normally in segmentation, its constituent cells being of about the size and structure of the cells of normal vesicles taken the early part of the seventh day after insemination. The cell-mass encloses a relatively small cavity which may be regarded as an abnormally placed segmentation cavity, in that its position is not eccentric, and that it is surrounded on all sides by more than one layer of cells. There is thus no differentiation of floor and roof as in normal blastodermic vesicles, and no development of ectoplacental cone and egg-cylinder as in the other ova obtained from

this uterus. I am for the present unable to offer any plausible explanation or give reasons for such abnormal development of the segmentation cavity. The fate of such a structure may perhaps be conjectured from a study of the abnormal ovum shown in A of figure 6, interpreted as showing a similar abnormality, but obtained in early stages of degeneration. This ovum and that shown in B of the same figure was obtained from the uterus of rat No. 90, 6 days, 17 hours, after insemination. In the uterus of this rat there are found six ova, only one of which was developed to a stage comparable to that shown in figure 24 (Part I) of about the same age. Three other vesicles present a slightly younger stage and may be compared with vesicles shown in D and E of figure 23, Part I. None of these four vesicles is favorably cut, but so far as may be determined, are of normal structure for the respective stages represented. A of figure 6 is also cut slightly obliquely, not sufficiently so, however, to make difficult its interpretation. The figure drawn is that of the third of a series of seven sections having $10\ \mu$ thickness, and depicts what is regarded as representing an ovum with abnormal segmentation cavity formation. In this ovum, the segmentation cavity is slightly more eccentric than is that shown in figure 5, and contains a granular detritus which in the preparations is distinctly stained with Congo red. The roof of this vesicle is composed almost throughout of more than one layer of cells. There is no differentiation of ectoplacental cone and ectodermal node, nor of yolk entoderm. Two cells regarded as phagocytic leucocytes, staining much more deeply in Congo red than do the cells of the ovum, have, in the section figured, penetrated the egg-mass, indicating early degenerative changes.

The vesicle shown in B of figure 6, obtained from the same rat, is favorably cut, and is readily followed through the series. The structural appearance presented by this vesicle is not explained by supposing it due to very oblique plane of section of a normal vesicle, a plane of section which might include the roof of the vesicle while avoiding its floor. The vesicle is abnormal in that it presents a want of development of the thickened germ disc, and a hyperdevelopment of the yolk entoderm. In none of the

sections of the series which includes this vesicle, which is cut in very favorable longitudinal direction, and is thus readily oriented with reference to mesometrial and antimesometrial portion, is there seen any thickening of the outer layer of cells, to form the part known as the floor of the vesicle, which at this stage of development is uniformly directed toward the mesometrial border. In A and B of figure 23, Part I, are shown vesicles with which the ovum here discussed may be compared. In the preparation under discussion, the yolk and parietal entoderm form almost a continuous layer, one of the detached cells showing a mitotic phase. In the normal vesicles of this stage of development the parietal entoderm is represented by a few scattered cells, as may be observed by a study of the figures to which reference is above made. Whether this vesicle is to be regarded as showing a later stage of an ovum in which there was irregularity in the formation of the segmentation cavity, I must for the present, leave as problematic. It has occurred to me that by enlargement of the segmentation cavity of an ovum such as shown in figure 5, with centrally placed segmentation cavity, there might result in further development the formation of a vesicle such as shown in B of figure 6.

It is freely admitted that the deductions here made, relative to irregularity in the formation of segmentation cavity, are not supported by conclusive evidence. It has seemed to me, however, that the interpretations given to the appearances presented are less open to criticism than others that might be suggested. These abnormal ova also suggest an inherent defect in the ova, leading to abnormal development, rather than abnormal development resulting from defective environment.

DEGENERATION OF OVA AS RESULT OF PATHOLOGIC UTERINE MUCOSA

In figure 7 are reproduced two ova which seem to me to show the primary stages of degeneration owing to pathologic condition of the uterine mucosa. Vesicle A was taken from the uterus of rat No. 91, 5 days, 16 hours, after insemination. In the uterus of this rat there were found only two ova. Vesicle B was taken

from rat No. 104, 6 days after insemination. In the uterus of this rat there were found six ova. In both of these rats, the ova present essentially the same stage of development, comparable to that shown in A and B of figure 23, Part I. As may be observed from the text of Part I (page 301) the stages obtained at the end of the sixth day and early hours of the seventh day, were found very difficult to fix. At this stage the ovum consists of a relatively large, thin walled vesicle, very prone to fixation shrinkage. All of the ova or vesicles obtained from rats Nos. 91 and 104, are very badly folded in their roof portion. Those shown



Fig. 7 Two ova of the albino rat partly surrounded by maternal blood with many phagocytic leucocytes. The folding of the roof of the vesicles is due to fixation shrinkage. $\times 200$. A, rat No. 91, 5 days, 16 hours, after the beginning of insemination. B, rat No. 104, 6 days after the beginning of insemination.

in A and B, figure 7, are representative. This folding, a result of imperfect fixation, is present in all of the vesicles of this stage, even though the respective vesicles present normal structure. The ova here figured may be regarded as having fairly normal structure, both as to rate of development and as to arrangement, form, and structure of constituent cells. All of the eight vesicles obtained from these two rats (No. 91, 2 ova; No. 104, 6 ova) are in part surrounded by exudated maternal blood, containing numerous leucocytes. Small masses of blood with leucocytes are found here and there in different parts of the uterine lumen of both rats, lodged in mucosal folds other than the characteristic decidual crypts enclosing the respective ova. These

decidual crypts are relatively shallow when compared with those of normal uteri of similar stages with normal ova. The uterine mucosa of the two rats under discussion does not appear to have reacted in a normal manner. In these preparations, attention is especially drawn to the presence of maternal blood with numerous phagocytic leucocytes found in relation with the ova, a condition never observed in normal development of ova and uterine mucosa. In A and B, figure 7, the red and white blood cells with granular detritus may be observed as found in relation with the respective vesicles, these presenting essentially the same appearances as do the other six ova obtained from these two rats; the one figured having been more favorably cut than any of the others. The appearances presented in these two rats are interpreted as showing a probable degeneration of the eight ova, and probably complete dissolution and removal. The vesicles appear to have developed normally to the stage at which they were obtained. As a result, however, of pathologic condition of the uterine mucosa, maternal blood, especially leucocytes, have entered the lumen of the uterus, the leucocytes being destined to play the rôle of phagocytes. In normal development of the albino rat, maternal blood does not enter the lumen of the uterus—decidual crypts—until after the uterine epithelium has become detached from the mucosa of the wall of the decidual crypt, in the region of lodgment of the enclosed ovum. Normally, very few leucocytes are met with in the lumen of the uterus, even in later stages of development, stages in which maternal red blood cells are met with in the decidual crypts. After experience had accumulated, uterine tubes supposed to contain developmental stages aging from the fourth to the sixth day, which on examination revealed blood and especially leucocytes in the lumen of the uterus, were regarded as not favorable specimens for finding ova. In a number of such uteri, cut completely in serial sections, no ova were found. It is possible that, owing to phagocytic action of the leucocytes present, the ova may have been completely removed prior to killing and fixing the tissues. In such condition, it would seem to me as pertinent to speak of faulty implantation, due to abnormal uterine mucosa. It seems to me signifi-

cant that in the two rats in which the pathologic condition affects primarily the maternal tissue, the uterine mucosa, all of the contained ova are prone to degeneration. In the abnormal ova previously described, for which it was suggested that the causes for the abnormality were to be sought in the ova themselves, in the great majority of instances, only one abnormal ovum was found in each uterus along with a variable number of ova which are to be regarded as normal for the respective stage.

IMPERFECT DEVELOPMENT OF THE ECTODERMAL VESICLE

The series contains two ova, very favorably cut, ova in which the ectodermal vesicle with the antimesometrial portion of the proamniotic cavity does not seem to have developed normally. Stages showing the differentiation of the egg-cylinder, the formation of the ectodermal vesicle with the antimesometrial portion of the proamniotic cavity, the formation of the mesometrial portion of the proamniotic cavity in the extraembryonic ectoderm, the union of the two primary proamniotic cavities to form a single space, are clearly shown in figures 26 and 27, Part I, in the series of closely approximated stages there portrayed. From a study of these figures, it will be observed that the antimesometrial portion of the proamniotic cavity develops within the ectodermal node before the mesometrial portion of this cavity develops in the extraembryonic ectodermal portion of the egg-cylinder. In the egg-cylinder shown in figure 8, rat No. 94, 8 days after the beginning of insemination, such is not the case. In the uterus of this rat there were found seven egg-cylinders, one of which, very favorably cut, is shown in C, figure 27, Part I. The other egg-cylinders obtained from this uterus, except the abnormally developed one to be discussed, though not favorably cut, present essentially the same form and structure as that figured under C of the figure above referred to. The egg-cylinder portrayed in figure 8 compares in size and form with those regarded as normal and taken from the same uterus. For the greater part it presents normal structure and normal relations of cells. The ectoplacental cone, only in part included in the figure, and the parietal

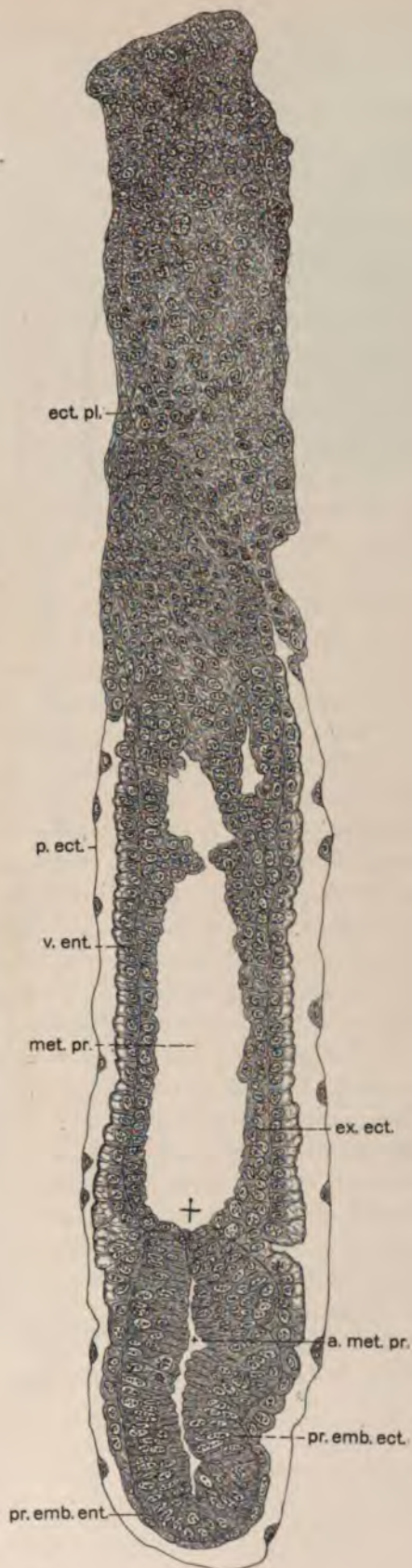


Fig. 8 Egg-cylinder of albino rat showing retarded development of ectodermal node and of the formation of the antimesometrial portion of the proamniotic cavity. $\times 200$. Rat No. 94, 8 days after the beginning of insemination. *ect.pl.*, ectoplacental cone or Träger; *v.ent.*, visceral entoderm; *met.pr.*, mesometrial portion of the proamniotic cavity; *p.ect.*, parietal or transitory ectoderm; *pr.emb.ent.*, primary embryonic entoderm; *ect.n.*, ectodermal node; *a.met.pr.*, imperfectly developed antimesometrial portion of proamniotic cavity; *ex.ect.*, extraembryonic ectoderm.

ectoderm, in structure and relation to decidual crypt, are to be regarded as of normal development. The visceral entoderm, surrounding the extraembryonic ectodermal portion of the egg-cylinder, is of normal structure, showing the three zones evidenc-

ing its absorptive function. The extraembryonic ectoderm, enclosing the mesometrial portion of the proamniotic cavity, presents normal structure and relations of cells. The only abnormality observed is in the region of the ectodermal node, the anlage of the ectodermal vesicle with the enclosed antimesometrial portion of the proamniotic cavity. With this stage of development of the egg-cylinder (see figs. 26 and 27, Part I) the ectodermal node presents a well formed cavity, surrounded by the cells of the primary embryonic ectoderm, radially arranged. In the egg-cylinder under discussion (fig. 8) there is distinctly a retardation in the development of the ectodermal vesicle with full differentiation of the primary embryonic ectoderm. An imperfectly developed antimesometrial portion of the proamniotic cavity is evident. This small cavity, indistinctly bounded, extends obliquely through several sections of the ectodermal node, and contains amorphous granular detritus, which in the preparations is stained by Congo red. The cells destined to form the primary embryonic ectoderm show no definite arrangement, especially as concerns the more centrally placed cells of the node. Since the primary embryonic ectoderm is the anlage for the ectoderm of the embryo, an arrest in its differentiation would of necessity profoundly affect further development of the embryo. Antimesometrial to the ectodermal node (just above it in the figure) there is found a small vesicle the walls of which are not distinctly delimited and composed of extraembryonic ectodermal cells, surrounding a small, completely bounded cavity. I am not prepared to say whether this small vesicle is to be regarded as developing from cells of the extraembryonic ectoderm, or from a displaced, accessory ectodermal node, in which a discrete portion of the proamniotic cavity has developed. If the latter, the possibility of a double anlage for the embryonic ectoderm is to be considered. My interpretation of this egg-cylinder as showing a retardation of the development of the ectodermal node and differentiation of the primary embryonic ectoderm, is confirmed from a study of a slightly older stage showing essentially the same condition. This ovum is presented in figure 9, and is taken from rat No. 41, 8 days, 16 hours, after the

Fig. 9 Egg-cylinder of albino rat, in which the antimesometrial and mesometrial portions of the proamniotic cavity have failed to unite to form a single or definite proamniotic cavity. $\times 200$. Rat No. 41, 8 days, 16 hours, after the beginning of insemination. *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *v.ent.*, visceral entoderm; *met.pr.*, mesometrial portion of the proamniotic cavity; *ex.ect.*, extraembryonic ectoderm; *a.met.pr.*, antimesometrial portion of the proamniotic cavity; *pr.emb.ect.*, primary embryonic ectoderm; +, region at which, in normal development, by the end of the eighth and beginning of the ninth day, the two portions of the proamniotic cavity would have united to form a single space, the definite proamniotic cavity.



beginning of insemination. The uterus of this rat contains eight egg-cylinders, all of which, except the one here figured, show normal structure, though presenting quite different stages of development. One of these, cut serially in cross-section, is figured in C, figure 32, Part I, as showing anlage of mesoderm with primitive streak and groove. Two of the other egg-cylinders show the anlage of the mesoderm, two others show late pre-mesoderm stages of the egg-cylinder, the remaining egg-cylinders are less fully developed, one showing a development which may be compared to B of figure 26, Part I, thus a much younger stage. By the end of the eighth day and with the early hours of the ninth day after the beginning of insemination in the albino rat, the two parts of the proamniotic cavity, which develop discretely, have joined to form a single space (C, fig. 27, Part I). The egg-cylinder shown in figure 9, presents normal development in all parts, except that there is as yet no union of the two parts of the proamniotic cavity. This egg-cylinder is most favorably cut, in longitudinal direction; the plane of section being almost parallel to the mid-sagittal plane. This egg-cylinder, therefore, is easily followed through the several sections of the series into which it was cut. The irregularity of outline of the ectodermal vesicle, lower right of figure, it is believed, is not due to fixation shrinkage. Judging from size and structural differentiation of this egg-cylinder, union of the antimesometrial and mesometrial portions of the proamniotic cavity should have been completed before this stage of development was reached, with the primary embryonic ectoderm and the extraembryonic ectoderm forming a continuous layer, as shown in figure 29, Part I. The folding of the wall of the antimesometrial portion of the egg-cylinder, lower right of figure, evident in nearly all of the sections of the series, is regarded as indicating an abnormal growth of the primary embryonic ectodermal cells composing the wall of the ectodermal vesicle, as a result of retarded extension of the antimesometrial portion of the proamniotic cavity, perhaps an adjustment to meet the altered mechanical stress resulting from abnormal development. The condition here seen, it would seem, is foreshadowed in the egg-cylinder shown in figure 8.

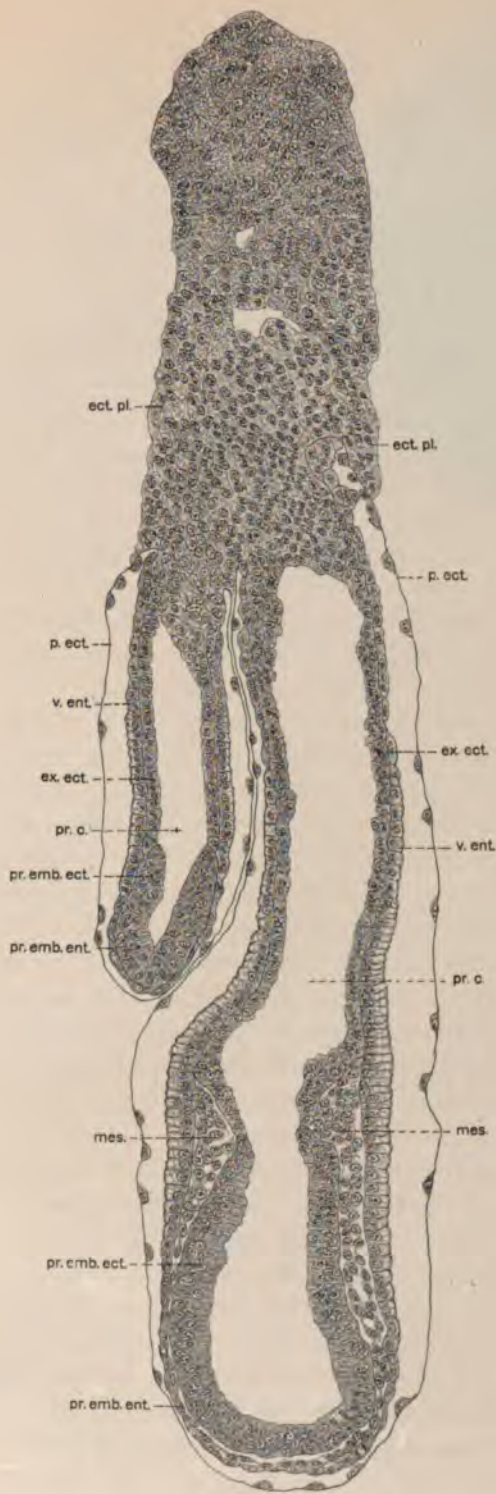


Fig. 10 Two egg cylinders of the albino rat found within the same decidual crypt, with in part common ectoplacental cone. $\times 150$. Rat No. 87, 9 days after the beginning of insemination. *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *v.ent.*, visceral entoderm; *ex.ect.*, extra-embryonic ectoderm; *pr.c.*, proamniotic cavity; *pr.emb.ect.*, primary embryonic ectoderm; *pr.emb.ent.*, primary embryonic entoderm; *mes.*, mesoderm.

The causes operative in this retardation of development and differentiation of the ectodermal vesicle and primary embryonic ectoderm, I have been unable to determine. They would appear to be inherent in the egg-cylinder, since ectoplacental cone and visceral entoderm, so far as may be determined from a study of sections, appear to have functioned normally, in furnishing the necessary embryotroph in the form of maternal hemoglobin, as is normal for egg-cylinders of the albino rat of this stage of development.

TWO EGG-CYLINDERS IN ONE DECIDUAL CRYPT

The ova portrayed in figure 10 present a condition which must be regarded as exceedingly rare, since it represents the only instance of this condition observed in the extended series of preparations of the various stages of the development of the albino rat from the end of the first to the end of the ninth day after insemination, in my possession. This preparation is from rat No. 87, 9 days after the beginning of insemination. The uterus of this rat contained, other than the preparation here considered, six egg-cylinders of normal development, all showing a stage which is slightly older than that shown in figure 31, Part I, in that the mesoderm shows further development than is shown in that figure. In the preparation here figured there are found two egg-cylinders enclosed within the same decidual crypt. This figure, which is drawn by combining the drawings made from two sections, is reproduced at a magnification of 150 diameters, while all of the other figures portraying sections of ova, both in Part I and in Part II of this communication, are reproduced at a magnification of 200 diameters. This should be borne in mind when comparing this figure with the others. In figure 10, the lower portion of the large egg-cylinder to the level of the lower end of the smaller one was drawn from one section, while the remainder of the figure was drawn from the fourth following one. The adjustment was made by overlapping in the camera lucida drawing ($\times 600$) the sharp mesometrial border of the primary embryonic ectoderm of the larger egg-cylinder. Scarcely any

adjustment was found necessary, none of the right wall of the larger egg-cylinder, and only very slightly so of its left wall. The slight deviation from the longitudinal axis of the larger egg-cylinder made the procedure desirable. It is thought that the figure as presented gives correctly the size of the respective egg-cylinders, and in all essentials, their relations; the greater part of the figure having been drawn from one section. Both of the egg-cylinders reveal normal structure for the stages of development attained. The larger one is cut in the coronal plane, as is readily determined by the distribution of the mesoderm, one side representing a mirror picture of the other. The direction of section in the smaller egg-cylinder, except that it is longitudinal, is not to be determined, since before the anlage of the mesoderm, a bilateral symmetry cannot be recognized in sections. Since these two egg-cylinders are in all essentials of normal form and structure, and since their structure is clearly brought out in the figure, an extended description of them at this place seems uncalled for. For respective stages the reader is referred to Part I. Attention may be drawn, however, to the fact that the visceral entoderm on the contiguous surfaces of the two egg-cylinders is less fully differentiated, and shows less absorption of the maternal hemoglobin than is seen on the exposed or free surfaces, this, no doubt, for mechanical reasons. Further, that in the region where the two egg-cylinders are in contact, the parietal ectoderm of each can be traced as a distinct layer to the bases of the respective ectoplacental cones, showing that each developed from a separate ovum. The ectoplacental cones are for a short distance distinct. In tracing the sections through the series the impression is gained that the ectoplacental cone of one of the egg-cylinders overlaps that of the other in such a way that in the plane of the sections obtained, one seems continuous with the other, as represented in the figure. The boundary between the two is not distinct, and it would seem that as a result of pressure, partial fusion of the two had taken place. The presence of two egg-cylinders, enclosed within a single decidual crypt, as shown in this figure, with one of them having much smaller size and representing a younger stage of development, I believe is

not to be explained on the supposition of superfecundation or superfoetation. The record for this rat does not show insemination on successive days. At The Wistar Institute, after all of the supposedly successful matings of albino rats, the females rats are caged apart from the males. The smaller egg-cylinder, though appreciably smaller, is in stage of development separated from the other by a time interval of perhaps less than 24 hours. It presents a stage of development which is comparable to C of figure 27 (8 days) and except for size, to the one figured in figure 29 (8 days, 17 hours) of Part I. It is believed that in this case both ova were seminated at about the same time, and proceeded through normal segmentation and that on reaching the lumen of the uterus during the fifth day they became lodged in close proximity in the same mucosal fold. With the development of the decidual crypts, both became enclosed within the same crypt, at perhaps slightly different levels. In further development one blastodermic vesicle dominated the other and from about the seventh day on, one developed and differentiated more rapidly than the other. Had development continued, two distinct embryos, with separate amniotic cavities, attached to the same placenta, would have been formed, with one embryo large and more fully developed than the other. From mere difference in size and of development of embryos in the same litter it is not warranted to postulate superfecundation nor superfoetation. I am of the opinion that usually when two morula masses are lodged in close proximity in the same mucosal fold, one or the other degenerates (fig. 2, A) and that the normal development of both, as in the preparation shown in figure 10, is of very rare occurrence.

CONCLUSIONS

A study of the abnormal or pathologic ova met with in the extended series of preparations covering the first ten days of the development of the albino rat, enables grouping them in two main classes:

a. Such in which all of the ova of a given rat show, or are associated with, abnormal development.

b. Such in which a single abnormal or pathologic ovum is found in the same uterus along with an average number of normally developed ova.

When all the ova in a given uterus show abnormality, the presumption seems warranted that the underlying cause of the abnormality is to be sought in an altered or pathologic condition of the uterine mucosa. In the instances observed, the presence of maternal blood with many phagocytic leucocytes was noted in the lumen of the uterus, adhering to and surrounding the ova. From the study of sections of the uteri of an appreciable number of albino rats, in which insemination and supposedly semination seemed normal, but in which on complete serial sectioning of the uterine tubes no ova were found, but in the lumen of the uterine tubes of which the presence of maternal blood and phagocytic leucocytes was noted, the conclusion seems warranted that death and complete absorption of ova, after a given stage of normal development has been reached, may occur. In such cases, one may with propriety speak of faulty implantation, due to altered or pathologic condition of the uterine mucosa, even in cases where no actual implantation would have occurred in corresponding normal stages. In the two rats (Nos. 91 and 104) in which this condition was observed, the decidual crypts were shallow and not developed to the extent normal for the respective stages, evidencing the abnormal condition of the mucosa.

In cases in which a single abnormal or pathologic ovum is found in the uterus along with several normal ova, the presumption seems justified that the underlying cause responsible for the abnormal development is to be sought in the ovum itself, and not in its environs.

Abnormal developmental stages, interpreted as due to irregular or retarded segmentation, irregular or abnormal segmentation cavity formation, and retarded development of the ectodermal node and primary embryonic ectoderm, where only a single ovum shows abnormal development in a uterus containing the average number of ova presenting normal development, are difficult to explain on the assumption that extraneous influences affecting a single ovum are operative. Practically all

of the abnormal ova of the class described, and especially is this true for older stages, present normal relations to the uterine mucosa and the walls of the decidual crypt after implantation, and so far as may be determined by structure, give evidence of normal absorption of maternal hemoglobin in stages in which such absorption is pertinent. It may be argued that a single ovum may be less favorably placed in relation to embryotroph or pabulum, and as a result of unfavorable nutrition, develop abnormally. This is difficult to conceive for stages in which the ova lie free in the lumen of the uterus, namely, to about the beginning of the seventh day after the beginning of insemination, when embryotroph or pabulum must be relatively evenly distributed. The presumption, it would seem to me, in such cases is in favor of regarding the primary cause of the abnormal development as inherent in the ovum.

Separation of the first two blastomeres and the presence of two egg-cylinders in a single decidual crypt are regarded as chance findings and as of rare occurrence, since each was met with only once in the material at hand.

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Literature on pathologic ova of the albino rat is lacking. For the literature of all but the more recent work, dealing with comparative experimental teratology, the bibliographies accompanying the chapters of O. and R. Hertwig may be consulted; for that dealing with the pathology of human ova, the bibliographies accompanying the contributions of F. P. Mall may be consulted.

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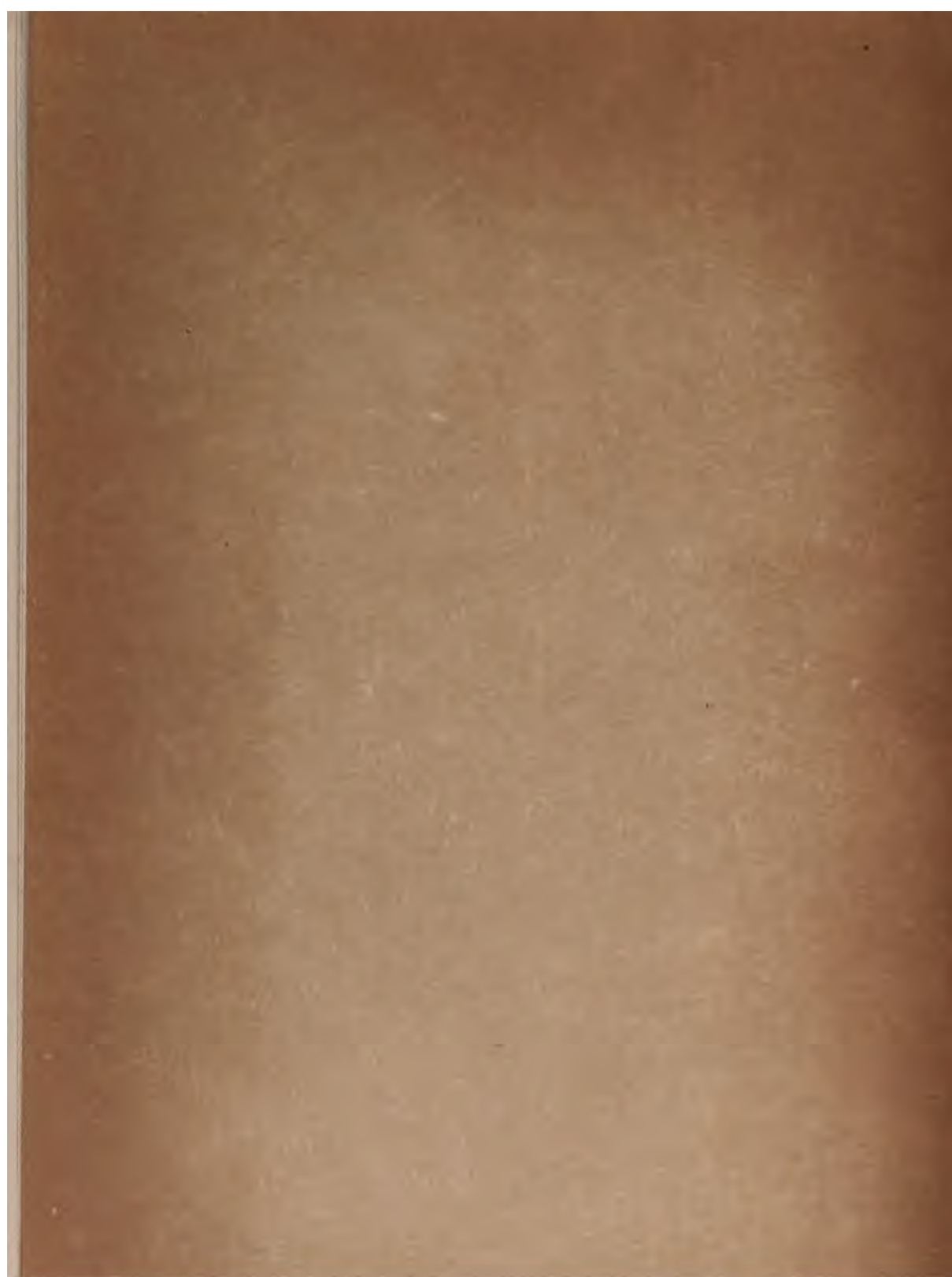
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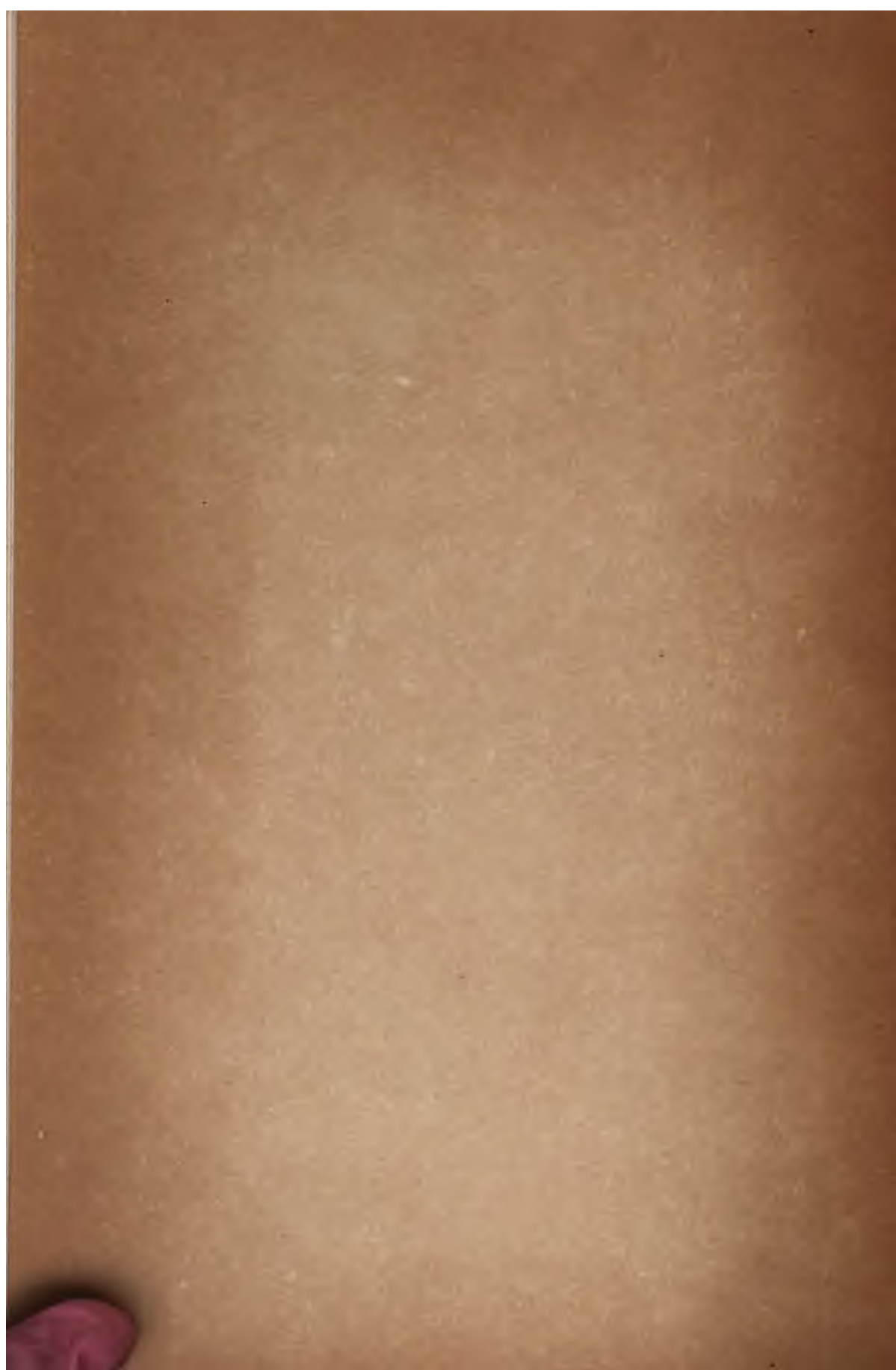
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